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INFLUENCES OF TROPICAL TREE DIVERSITY ON TREE ROOT DECOMPOSITION

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INFLUENCES DE LA DIVERSITÉ DES ARBRES TROPICAUX SUR LA DÉCOMPOSITION DES RACINES DES ARBRES

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PRÉSENTÉ

COMME EXIGENCE PARTIELLE

DE LA MAÎTRISE EN BIOLOGIE

BY

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Siempre has confiado en mí, hasta en los momentos que ni yo creía, has sido mi motivación y el recuerdo de tu perseverancia hace que en los momentos que estoy por perder la esperanza, recupere la fuerza y siga luchando. A pesar de la distancia que implicaba perseguir mis sueños, estuviste hay para ayudarme a dar el paso, para hacerme respirar profundo, secar mis lagrimas y dejarme volar. Por esa gran mujer que llevo en mi corazón, la que me ayudo a ser la mujer que soy, personal y profesionalmente, este es nuestro nuevo triunfo y es todo para y por ti, mamá.

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°C	Degree Celsius
min	Minutes
h	Hour
mg	Milligram
g	Gram
kg	Kilogram
mL	Milliliter
m	Meter
cm	Centimeter
mm	Millimeter
M	Molar
mM	Millimolar
µM	Micromolar
nm	Nanomolar
µmol	Micromole
%	Percent
log	Logarithmic
V	Velocity of reaction
Km	Michaelis constant
S	Substrate concentration
k	Decomposition rate
AG	α-glucosidase
ßG	ß-glucosidase
CEL	Cellobiohydrolase
XYL	ß-xylanase
NAG	*N*-acetyl-ß-D-glucosaminidase
PME	Phosphomonoesterase
AS	Aryl sulphatase

SRL	Specific root lengh
RDMC	Root dry matter content
NDF	Neutral detergent fiber
ADF	Acid detergent fiber
HemiCell	Hemicellulose
Cell	Cellulose
C	Carbon
N	Nitrogen
P	Phosphorus
Al	Aluminum
B	Boron
Ca	Calcium
Cu	Copper
Fe	Iron
NaN_3	Sodium azide
NaOH	Sodium hydroxide
MU	4-methylumbelliferyl

1.1 Résumé

Les processus écologiques à l'échelle de l'écosystème, comme la décomposition, peuvent être affectés par des modifications dans la composition des arbres du couvert forestier résultant du climat et de l'usage des terres au sein des écosystèmes tropicaux. Le principal objectif de ce premier chapitre était d'examiner l'influence de la diversité des arbres tropicaux sur la décomposition de racines à deux échelles spatiales distinctes. Ceci a été réalisé en mesurant les taux de décomposition et l'activité enzymatique des décomposeurs à l'échelle de l'unité d'échantillonnage (sac de litière 0.002m²) ou á l'échelle de la parcelle (taille de parcelle 2000m²), soit deux échelles et qui sont liées directement (transfert de nutriments) ou indirectement (microclimat) à la diversité des arbres. Nous avons également évalué 1) comment, à l'échelle de l'unité d'échantillonnage, les effets nets de la diversité seraient influencés par l'hétérogénéité biotique et abiotique et 2) à quel point la variation interspécifique des traits fonctionnels racinaires des arbres tropicaux explique la décomposition racinaire grossière. Les sacs de litière ont été disposés sur deux sites au Panama, Sardinilla et Agua Salud caractérisés par des propriétés de sol contrastées. Les sacs de litière comprenant différents mélanges d'espèces ont été placés pour décomposition sous couvert forestier soit de faible ou de forte diversité. L'influence de la diversité des arbres, à l'échelle de l'unité d'échantillonnage et de la parcelle, a été étudiée en plaçant une espèce seule ou cinq espèces locales d'arbres tropicaux mélangés dans différentes conditions de diversité du couvert forestier. Les sacs de litière ont été mis à décomposer en juillet 2011 et collectés après 50, 160/195 (Agua Salud/Sardinilla), 310, et485 jours afin de déterminer les taux de décomposition en utilisant la perte en masse. En plus, l'activité des enzymes impliqués dans les cycles du carbone, de l'azote et du phosphore a été mesurée après 485 jours de décomposition. Nos résultats ont montré que la diversité des arbres n'influence pas significativement la décomposition des racines, et ce peu importe l'échelle spatiale étudiée. Cependant, une importante variance des réponses à l'échelle de l'unité d'échantillonnage a été observée, suggérant une forte hétérogénéité spatiale. L'importance des effets du site, surtout associés aux caractéristiques du sol ainsi que la densité des espèces de décomposeurs de racines ont contribué à une variation intraspécifique des taux de décomposition. Les variations interspécifiques des traits racinaires expliquaient entre 35 (Sardinilla) et 80% (Agua Salud) des taux de décomposition des racines. Notre étude suggère que les changements en termes de décomposition ne sont pas uniquement influencés par la diversité des arbres du couvert forestier mais plutôt par des changements dans la composition fonctionnelle des forêts tropicales qui peuvent altérer la quantité et la qualité des entrées de litières racinaires.

Mots-clés: racine, effets de diversité, processus souterrain, échelles spatiales, activités enzymatiques, sol, traits fonctionnels racinaires, cycle des nutriments, Panama.

1.2 Abstract

Ecosystem-level ecological processes, such as decomposition, may be affected by changes in tree overstory composition that may result from climate and land-use change in tropical ecosystems. Thus, our objective in this chapter was to examine the influence of diversity of tropical trees on coarse root decomposition by measuring decomposition rates and enzymatic activity at two spatial scales, micro- (within bag: $0.02m^2$) and meso- (within plot: 2000 m^2) scales, that are related to direct, e.g. nutrient transfer, and indirect effects, e.g. microclimate, of tree diversity on this ecosystem process, respectively. As well, we evaluated: 1) how net diversity effects at the micro-scale could be affected by abiotic and biotic heterogeneity and 2) the extent to which interspecific variation in root functional traits of tropical species explained coarse root decomposition. We established coarse root decomposition bags that varied in species composition in two sites in central Panama with contrasting soil properties, Sardinilla and Agua Salud. The influence of tree diversity at both spatial scales was examined using tree overstory plots of either low or high diversity (meso-scale) and decomposition bags with low, i.e. single-species coarse roots, and high, i.e. a five species coarse root mixture, diversity levels (micro-scale). Decomposition bags were established in July 2011 and collected after 50, 160/195 (Agua Salud/Sardinilla), 310, and 485 days to determine decomposition rates using mass loss. In addition, activity of enzymes involved in carbon, nitrogen, and phosphorus cycles were measured after 485 days of decomposition. Our results showed that tree diversity did not significantly influence coarse root decomposition and enzymatic activity at either of the studied spatial scales. However, considerable variation of net diversity effects at the micro-scale was observed, suggesting a strong effect of environmental heterogeneity. The importance of site effects, predominantly differences in soil characteristics, and species identity of the decomposer root material contributed to intraspecific variation in decomposition rates. Interspecific variation in root traits explained 35 and 80 % of variation in coarse root decomposition rates in Sardinilla and Agua Salud, respectively. Our study suggests that changes in decomposition is not mediated by tree overstory diversity per se, but rather by changes in the functional composition of tropical forests that may alter the quantity and quality of root litter input.

Keywords: coarse roots, diversity effects, below-ground processes, spatial scales, enzymatic activities, soil, root functional traits, nutrient cycle, Panama

GENERAL INTRODUCTION

Organic matter decomposition is the reciprocal process of primary plant production in terrestrial ecosystems, whereby assimilated carbon retained in structural plant biomass is transferred to the soil as leaf, wood, or root litter and released by decomposer communities (Malhi and Grace 2000, Gessner et al. 2010). Studies in boreal, temperate, and tropical forests report that large portions of assimilated carbon are transferred belowground (Vogt et al. 1986, Grayston et al. 1996, Malhi et al. 1999), yet little remains known about the turnover of belowground components, such as coarse roots of trees, particularly in tropical regions (Giardina et al. 2005, Meister et al. 2012). Tropical forests play an important role in the global nutrient cycle (Vitousek and Sanford 1986, Malhi et al. 1999) to which they contribute ~70 % of terrestrial nitrogen fixation (Townsend et al. 2011) and stock an estimated 40 % of terrestrial carbon (Soepadmo 1993). However, nutrient cycles in tropical ecosystems are being altered by land use change (Foley et al. 2003, Townsend et al. 2011), principally through the transformation of vegetations covers, e.g. forest to agricultural and pasture fields, and the establishment of native or exotic tree plantations (Achard et al. 2002, Lambin et al. 2003, Piotto et al. 2010), bringing uncertainty to global estimates of terrestrial carbon. This thesis aims to improve our knowledge of coarse root decomposition in tropical forests in the context of multi-species plantations with the hope of improving our estimates of this component of the carbon cycle for global models.

Despite their important contribution to the nutrient cycle in terrestrial ecosystems, belowground processes have been less studied than aboveground processes, mostly due to logistical challenges. Belowground carbon could be underestimated by as much as 40 % globally (Robinson 2007), increasing estimates of belowground carbon in tropical forests from 74 to 123 Pg and total tropical forest carbon stocks from 553 to 602 Pg (Robinson 2007). In a meta-analysis on global patterns of root turnover that included graminoids, shrubs and fine tree roots, Gill and Jackson (2000) found a steady increase with decreasing latitude in annual turnover of maximum root biomass ranging

on average from 13% in boreal ecosystems to 73% in tropical ecosystems. When contrasting tree root diameter classes, fine root litter had an annual turnover rate of 56%, while larger tree root sizes had an annual turnover rate of only 10%, exhibiting considerable variation (Gill and Jackson 2000). Across ecosystems globally, the fastest decomposition rates of aggregated litter types was found in tropical rain forests with 1.3 g g^{-1} yr^{-1} and the slowest rates were found in tundra with 0.18 g g^{-1} yr^{-1} (Zhang et al. 2008). However, only seven studies on root decomposition were included in the meta-analysis, while for other groups, such as broad-leaved litter, as many as 154 studies were included (Zhang et al. 2008). Contributions of root turnover to the carbon cycle may be currently under- or overestimated simply due to the lack of available information.

To date, the dominant focus in decomposition studies has been on leaf litter and on the determination of which factors influence their decomposition rates, such as climatic conditions (Aerts 1997, Hättenschwiler et al. 2011), decomposer community composition (Schmidt et al. 2008, Gessner et al. 2010, Handa et al. submitted) and litter quality (Cornwell et al. 2008, Zhang et al. 2008). Whether these factors affect belowground decomposition similarly remains to be determined. In the meta-analysis by Silver and Miya (2001), studies on root decomposition were limited mostly to fine root decomposition in temperate forests and grasslands. Contrary to expectations, comparative studies on leaf litter and root litter decomposition have reported contradictory results (Cusack et al. 2009, Hobbie et al. 2010). Cusack et al. (2009) found no differences between leaf and root decomposition rates in a tropical forest; although different drivers explained their respective decomposition. Root decomposition was explained mainly by temperature and lignin concentration, while leaf decomposition by seasonality (Cusack et al. 2009). On the other hand, Hobbie et al. (2010) found that leaf decomposition rates were not correlated with root decomposition rates. One possible explanation for de-coupled leaf and root decomposition rates is related to litter quality (Silver and Miya 2001). The same species could have different leaf, wood, and root chemical composition, likely reflecting different physiological functions (Westoby and Wright 2006, Baraloto et al. 2010). Furthermore, different traits possibly influence

above and belowground decomposition across species, which would result in root and leaf decomposition rates with contrasting directions and magnitudes (Hobbie et al. 2010). In temperate tree species, fast leaf decomposition was associated with higher hemicellulose content and thinner leaves, while faster root decomposition was associated with higher calcium and lower lignin content (Hobbie et al. 2010).

The majority of studies on decomposition have been limited to single species leaf litter (Prescott 2010). Still, studies on decomposition of leaf litter mixtures from various biomes and ecosystems have highlighted the importance of considering species interactions in order to detect additive or non-additive effects of species composition on ecosystem function (e.g., Ball et al. 2008, Schindler and Gessner 2009, Gießelmann et al. 2010). Additive effects occur when the decomposition rate in mixtures is equal to the sum of litter decay rates of each species, while non-additive effects occur when the decomposition rate of the mixture is greater or less than the sum of decay rates of individual species (Gartner and Cardon 2004, Hättenschwiler et al. 2005). While additive effects have emphasized, in part, the importance of species identity and how species with divergent decomposition rates can cancel each other out when mixed together (Ball et al. 2008), non-additive effects have illustrated the importance of species interactions in litter mixtures, showing either synergistic (positive) or antagonistic (negative) effects on decomposition (Gartner and Cardon 2004). Using a plant community perspective enables diversity or species composition effects on decomposition to be evaluated (Hättenschwiler et al. 2005). However, studies where litter mixtures decompose in a 'common garden' type environment could limit the spatial scale at which diversity effects operate to the scale of the litter bag, as they exclude the potential influence of diversity of the surrounding plant community.

Decomposer communities, comprised of detritivores and microbial decomposers, play a vital role in the decomposition process (Gessner et al. 2010). Microbial decomposers break down lignocellulose and recycle detrital nutrients such as nitrogen, phosphorus, and sulfur making use of enzymes (Sinsabaugh et al. 1991,

Sinsabaugh et al. 2002). These enzymes are used to catalyze the degradation of plant biomass, breaking down complex chemical compounds into simpler compounds that are more readily assimilated (Sinsabaugh et al. 1991, Sinsabaugh et al. 2002). Enzymatic activity has been used as a biochemical measure of instantaneous degradation rates and as a robust indicator of microbial activity at different stages of degradation (Sinsabaugh et al. 1991, Sinsabaugh et al. 2002). Given the variation of enzymatic activity across decomposing plant species (Allison and Vitousek 2004), measuring the activity of key enzymes involved in C, N and P degradation may help to understand the observed variation in decomposition rates in species-rich tropical forests.

This thesis presents two chapters that explore various factors that influence the decomposition of coarse roots in tropical forest plantations and a natural forest in central Panama. In the first chapter, the effects of tree diversity on tree root decomposition rates and enzymatic activity are studied at two spatial scales and in contrasting decomposition environments. More specifically, we ask 1) how does tree species diversity influence decomposition of tree roots at meso- scale (within plot: 2000 m^2), e.g. indirect effects of tree overstory on decomposer communities, micro-climate conditions and soil properties, and the decomposition at micro- scale (within bag: 0.02 m^2), e.g. direct effects due to nutrients transfer among litters and complementarity resources for decomposers and 2) will species functional identity, i.e. root functional traits, contribute to understanding root decomposition rates? In the second chapter, enzymatic activity, a method typically used in soil science, is adapted for use in the root decomposition study presented in the previous chapter for use as an indicator of the microbial decomposer community. Specifically, we ask 1) to what extent does the activity of particular enzymes sampled on decomposed roots explain changes in root decomposition over time and 2) is this enzymatic activity influenced by species composition of litter mixtures and decomposition environment?

This thesis attempts to offer several novel contributions to the scientific community. Firstly, few studies on tree diversity effects have focused on belowground

components such as coarse root decomposition and microbial decomposers, even less in tropical ecosystems. Secondly, in contrast to most studies on diversity effects on decomposition, the use of multiple spatial scales provides a more complete plant community perspective and the comparison of different forest sites allows for observing the influence of site-specific external factors on the diversity and root decomposition relationship (Chapter 1). Thirdly, we assess the relative importance of particular structural, chemical and anatomical functional traits that are related to root decomposition (Chapter 1). Given that root decomposition is a complex process, the exploration of contrasting and promising approaches that provide a link between plant components and microbial decomposer communities is required. The adaptation of the method of soil enzymatic activity to decomposed roots in our study (chapter 2) provides a fourth novel contribution where we describe clear parameters to quantify enzymatic activity on dead roots. We also show the relationship between root decomposition and various enzymes involved in the C, N, and P nutrient cycles over time and identify factors that could influence enzymatic activity during root decomposition (chapter 2).

To address these various questions, we conducted a root decomposition field experiment during 485 days using coarse roots from five native tropical tree species that encompass a broad range of life histories strategies. Roots decomposed in two tree overstory diversity levels, low or high diversity, at two sites, Sardinilla and Agua Salud, under different soil conditions. Two diversity levels were also used within root bags and consisted of either low (single species) or high (five species mixtures) diversity. A suite of various structural, chemical and anatomical functional traits was measured on all species. Enzymatic activity of key enzymes involved in C, N or P degradation were measured in the beginning, middle and at the end of the experiment in different root decomposition treatments. Collectively, we aim that these two chapters improve our understanding on the role of tree diversity for coarse root decomposition and decomposer activity in tropical forests.

CHAPTER I

INFLUENCES OF TREE DIVERSITY ON TROPICAL TREE COARSE ROOT DECOMPOSITION AT MICRO- AND MESO- SCALES

1.1 Résumé

Les processus écologiques à l'échelle de l'écosystème, comme la décomposition, peuvent être affectés par des modifications dans la composition des arbres du couvert forestier résultant du climat et de l'usage des terres au sein des écosystèmes tropicaux. Le principal objectif de ce premier chapitre était d'examiner l'influence de la diversité des arbres tropicaux sur la décomposition de racines à deux échelles spatiales distinctes. Ceci a été réalisé en mesurant les taux de décomposition et l'activité enzymatique des décomposeurs à l'échelle de l'unité d'échantillonnage (sac de litière 0.002m²) ou á l'échelle de la parcelle (taille de parcelle 2000m²), soit deux échelles et qui sont liées directement (transfert de nutriments) ou indirectement (microclimat) à la diversité des arbres. Nous avons également évalué 1) comment, à l'échelle de l'unité d'échantillonnage, les effets nets de la diversité seraient influencés par l'hétérogénéité biotique et abiotique et 2) à quel point la variation interspécifique des traits fonctionnels racinaires des arbres tropicaux explique la décomposition racinaire grossière. Les sacs de litière ont été disposés sur deux sites au Panama, Sardinilla et Agua Salud caractérisés par des propriétés de sol contrastées. Les sacs de litière comprenant différents mélanges d'espèces ont été placés pour décomposition sous couvert forestier soit de faible ou de forte diversité. L'influence de la diversité des arbres, à l'échelle de l'unité d'échantillonnage et de la parcelle, a été étudiée en plaçant une espèce seule ou cinq espèces locales d'arbres tropicaux mélangés dans différentes conditions de diversité du couvert forestier. Les sacs de litière ont été mis à décomposer en juillet 2011 et collectés après 50, 160/195 (Agua Salud/Sardinilla), 310, et485 jours afin de déterminer les taux de décomposition en utilisant la perte en masse. En plus, l'activité des enzymes impliqués dans les cycles du carbone, de l'azote et du phosphore a été mesurée après 485 jours de décomposition. Nos résultats ont montré que la diversité des arbres n'influence pas significativement la décomposition des racines, et ce peu importe l'échelle spatiale étudiée. Cependant, une importante variance des réponses à l'échelle de l'unité d'échantillonnage a été observée, suggérant une forte hétérogénéité spatiale. L'importance des effets du site, surtout associés aux caractéristiques du sol ainsi que la densité des espèces de décomposeurs de racines ont contribué à une variation intraspécifique des taux de décomposition. Les variations interspécifiques des traits racinaires expliquaient entre 35 (Sardinilla) et 80% (Agua Salud) des taux de décomposition des racines. Notre étude suggère que les changements en termes de décomposition ne sont pas uniquement influencés par la diversité des arbres du couvert forestier mais plutôt par des changements dans la composition fonctionnelle des forêts tropicales qui peuvent altérer la quantité et la qualité des entrées de litières racinaires.

Mots-clés: racine, effets de diversité, processus souterrain, échelles spatiales, activités enzymatiques, sol, traits fonctionnels racinaires, cycle des nutriments, Panama.

1.2 Abstract

Ecosystem-level ecological processes, such as decomposition, may be affected by changes in tree overstory composition that may result from climate and land-use change in tropical ecosystems. Thus, our objective in this chapter was to examine the influence of diversity of tropical trees on coarse root decomposition by measuring decomposition rates and enzymatic activity at two spatial scales, micro- (within bag: 0.02m^2) and meso- (within plot: 2000 m^2) scales, that are related to direct, e.g. nutrient transfer, and indirect effects, e.g. microclimate, of tree diversity on this ecosystem process, respectively. As well, we evaluated: 1) how net diversity effects at the micro-scale could be affected by abiotic and biotic heterogeneity and 2) the extent to which interspecific variation in root functional traits of tropical species explained coarse root decomposition. We established coarse root decomposition bags that varied in species composition in two sites in central Panama with contrasting soil properties, Sardinilla and Agua Salud. The influence of tree diversity at both spatial scales was examined using tree overstory plots of either low or high diversity (meso-scale) and decomposition bags with low, i.e. single-species coarse roots, and high, i.e. a five species coarse root mixture, diversity levels (micro-scale). Decomposition bags were established in July 2011 and collected after 50, 160/195 (Agua Salud/Sardinilla), 310, and 485 days to determine decomposition rates using mass loss. In addition, activity of enzymes involved in carbon, nitrogen, and phosphorus cycles were measured after 485 days of decomposition. Our results showed that tree diversity did not significantly influence coarse root decomposition and enzymatic activity at either of the studied spatial scales. However, considerable variation of net diversity effects at the micro-scale was observed, suggesting a strong effect of environmental heterogeneity. The importance of site effects, predominantly differences in soil characteristics, and species identity of the decomposer root material contributed to intraspecific variation in decomposition rates. Interspecific variation in root traits explained 35 and 80 % of variation in coarse root decomposition rates in Sardinilla and Agua Salud, respectively. Our study suggests that changes in decomposition is not mediated by tree overstory diversity per se, but rather by changes in the functional composition of tropical forests that may alter the quantity and quality of root litter input.

Keywords: coarse roots, diversity effects, below-ground processes, spatial scales, enzymatic activities, soil, root functional traits, nutrient cycle, Panama

1.3 Introduction

Globally, natural and anthropogenic climate and land-use change are altering drastically plant community diversity and composition. These alterations, mainly biodiversity loss, are affecting ecological processes such as productivity and decomposition and, hence, ecosystem functions (Cardinale et al. 2012, Hooper et al. 2012). Tropical ecosystems are rich in biodiversity and play a vital role in global nutrient cycles. These ecosystems are estimated to store 40% of global carbon (C) stocks, although belowground C stocks, which have been poorly characterized due to logistical constraints, are likely underestimated (Robinson 2007). Despite the importance of tropical forest ecosystems in terms of the global C cycle and biodiversity, there are relatively few studies that have examined diversity effects on belowground processes in these ecosystems.

Organic matter decomposition is driven by the litter quality of the decomposing substrate, abiotic factors of the decomposition environment and biotic factors such as the decomposer community diversity (Gessner et al. 2010). Decomposer communities, comprising of both meso- and macrofauna, as well as microbial decomposers, may also be affected by land-use change (Heijden et al. 2008). These organisms contribute to decomposition through different mechanisms. While macrodetritivores act as litter fragmenters making litter more accessible to smaller decomposers (David & Handa 2010), microbial communities degrade lignocellulose and recycle detrital nutrients using different enzymes that break down complex chemical compounds into simpler, more readily assimilated compounds (Sinsabaugh et al. 1991, Sinsabaugh et al. 2002). As a result of the diverse functions of contrasting enzymes during decomposition, enzymatic activity has been used as a good indicator of degradation (Sinsabaugh et al. 2002), varying markedly across plant species (Allison and Vitousek 2004) likely due to species-specific differences in traits that are related to litter quality.

Functional plant traits, which include chemical, structural and anatomical traits in plant litter, have been useful in general to understand ecosystem processes and to link

above- and belowground dynamics (Cusack et al. 2009, Hobbie et al. 2010, Birouste et al. 2011). Leaf traits that determine leaf litter quality, such as total phenolics and nonstructural carbohydrates, influence decomposition rates in tropical forests (Coq et al. 2010, Hättenschwiler et al. 2011). While studies on wood and root decomposition in the tropics are scarce, traits such as vessel area proportion and diameter have been found to be strong predictors of decomposition rates (Cusack et al. 2009, Geffen et al. 2010). Due to the importance of functional traits in processes like decomposition, diversity losses associated with particular traits could generate complex responses across ecosystems (Cardinale et al. 2012).

Tree overstory diversity may influence belowground properties and processes, as well as the surrounding plant community. The "home field advantage" hypothesis (Gholz et al. 2000) posits that a particular tree overstory composition can promote a decomposer community that is more efficient in breaking down local types of litter. However, evidence for this hypothesis remains limited. A recent reciprocal transplant experiment across multiple biomes showed that plant litter traits were more important than local decomposer communities in predicting decomposition rates (Makkonen et al. 2012). Species identity of the tree overstory also could influence soil biochemical processes directly by modifying the availability of resources, such as water, soil nutrients and light, to competitors while, at the same time improving their own fitness (tight weave hypothesis). Indirectly, tree overstory could change soil properties and processes, e.g. decomposition, through mechanisms that improve a species' own performance, such as the production of recalcitrant, flammable litter in response to herbivore pressure (loose weave hypothesis); or by affecting soil processes due to tree-soil interactions, e.g. mycorrhizal fungi (frayed hypothesis) (Binkley and Giardina 1998). In tropical forests, tree overstory composition affects soil processes such as carbon mineralization, phosphorus acquisition, litter quality input, and carbon pools (Potvin et al. 2011, Keller et al. 2013), e.g. soil carbon fluxes changes due to root exudations (Grayston et al. 1996). Despite the possible influences of tree overstory on decomposition, how overstory

diversity and composition may affect root decomposition in tropical forests remains unknown.

As decomposition in natural ecosystems is a product of litter comprised of co-occurring species in a particular plant community (Gartner and Cardon 2004, Hättenschwiler et al. 2005), the use of litter mixtures to evaluate diversity effects on decomposition has become increasingly frequent (Gartner and Cardon 2004). Comparisons of litter mixture decomposition rates with decomposition rates of individual species can be used to assess whether the effects of increasing species diversity is additive or non-additive. Additive effects on decomposition reflect the neutral result of mixing various species together (Gartner and Cardon 2004). Non-additive effects (referred to also as positive diversity effects) on litter mixtures can be explained by: 1) complementarity effects mediated by decomposer communities, e.g. litter mixtures create more diverse micro-habitats and, thus, change the abundance and activity of decomposers (Hansen and Coleman 1998, Gießelmann et al. 2010) and 2) nutrient transfer or presence of inhibitory components by leaching or biological mediation (Fyles and Fyles 1993, Schimel and Hättenschwiler 2007). Non-additive effects when observed, show in general a much weaker response in leaf litter decomposition experiments than what has been observed for plant productivity in biodiversity experiments (Handa et al. submitted). However, the spatial scale at which litter mixtures have been studied has been limited generally at the micro scale, i.e. the mechanisms related to interactions inside the litter bag listed (Fyles and Fyles 1993). Moreover, few studies make use of multi-site comparisons, which test the sensitivity of diversity effects on litter mixtures to environmental biotic and abiotic heterogeneity (Madritch and Cardinale 2007).

Diversity effects in natural ecosystems could vary across temporal and spatial scales (Duffy 2009). Across temporal scales, diversity effects on productivity change due to increasing species complementarity (Cardinale et al. 2007) and due to changes in the magnitude of negative and positive plant-soil feedbacks (Eisenhauer et al. 2012). Across

spatial scales, belowground processes could be affected by tree overstory compositions/diversity due to changes in the distribution of soil communities: soil microbial communities are affected by trees at fine scales, e.g. by root traits and exudation, by plant community composition at intermediate- scales, and by vegetation types at large scales (Ettema and Wardle 2002). In the tropics, evidence of diversity effects operating at extremely local spatial scales, i.e. few meters, has been found for productivity (Uriarte et al. 2004b, Potvin and Dutilleul 2009). Potvin and Dutilleul (2009) found that the individual tree productivity, i.e. tree diameter and height, was more strongly affected by the size of neighbors than by diversity. While the effects of neighbors on tree growth decreases with distance, the influence of the neighborhood depends strongly on the target species in tropical forests (Uriarte et al. 2004a, Uriarte et al. 2004b). In spite of the importance of spatial scales in explaining diversity effects on ecological processes in tropical ecosystems, the influence of diversity effects on belowground processes in the tropics, such as root decomposition, has yet to be determined. Hence, to understand if similar spatial scales that apply to productivity (Weigelt et al. 2007) also apply to decomposition in the tropics, we used a multi–scale approach to evaluate direct and indirect diversity on root decomposition.

Our study aimed to determine how the diversity of tropical trees influenced the decomposition rate of coarse roots and the enzymatic activity of decomposer communities at two spatial scales during 485 days. We tested a) the indirect effect of tree overstory diversity on decomposition at the meso- scale (within plot: 2000 m^2) and b) the direct effect of tree root diversity at micro- scale (within the decomposition bag: 0.02 m^2). At the meso- scale, we expected that where tree overstory species diversity was higher, there would be faster root decomposition rates due to positive, indirect effects on the abiotic decomposition environment (Prescott 2002, Chapin 2003, Keller et al. 2013). We also hypothesized that tree diversity at both scales would accelerate decomposition, mediated by the biotic decomposer and detritivore communities, due to complementary resource availability in mixtures (Hättenschwiler et al. 2005, Wardle 2006, Thoms et al. 2010, Eisenhauer 2012). Finally, we expected that

species functional identity based on root functional traits (Hobbie et al. 2010) would explain coarse root decomposition rates across sites, as found in leaf decomposition studies (Cornwell et al. 2008, Hättenschwiler et al. 2011, Freschet et al. 2012).

1.4 Methods

1.4.1 Study sites

The study was done in two experimental forest sites in central Panama, Sardinilla (9°19' N - 79°38' W) and Agua Salud (9°13' N - 79°47' W), with annual precipitations of 2350 mm and 2300 mm, respectively (Scherer-Lorenzen et al. 2007, Breugel et al. 2011). The Sardinilla site is an experimental diversity tree plantation established in 2001 where former pasture land was planted in plots of 45 x 45 m with either one, three or six species of native tree species (Scherer-Lorenzen. et al. 2005, Scherer-Lorenzen et al. 2007). The tree species used in the Sardinilla plantation were selected to have a gradient of relative growth rates (Table 1.1) of slow growing species, *Cedrela odorata* and *Tabebuia rosea,* moderate growing species, *Anacardium excelsum* and *Hura crepitans,* and fast growing species, *Cordia alliodora* and *Luehea seemanii* (Scherer-Lorenzen et al. 2007). Mortality of one species, *Cordia allidora*, was high in the early years such that it is no longer considered to be represented within the tree diversity treatments at the site (Potvin and Gotelli 2008). After 10 years of growth, the Sardinilla plantation has a closed, stratified canopy (Kunert et al. 2011). Understory vegetation is varied and is dominated by grasses and herbaceous species (Potvin et al. 2011). Soils originated from sedimentary rocks and tertiary limestone, presenting Alfisols dominated by clay (Potvin et al. 2004).

The Agua Salud forest sites include young native tree plantations established in 2008 on former cattle pastures and agricultural fields and secondary forests of different ages, ranging from one to eighty years old (Breugel and Hall 2008, Breugel et al. 2011, Paul et al. 2011). In contrast to Sardinilla, the Agua Salud plantation plots are 45 m x 39 m (Breugel and Hall 2008) and are characterized by short, steep slopes with soils derived from pretertiary basalt plateau (Hassler et al. 2011). A subset of the planted native tree

species (*Anacardium excelsum* and *Tabebuia rosea*) in Agua Salud also occur in Sardinilla. After three years of growth, the canopy remains open with a vigorous understory dominated by exotic pasture grasses, as most individuals are less than ~2.5 m in height.

Table 1.1 Ecological characteristics of the studied native tropical tree species.

Species	Family	Successional stage	Relative growth rate	Leaf phenology
Anacardium excelsum	Anacardiaceae	Light-intermediate	Intermediate	Evergreen
Cedrela odorata	Meliaceae	Shade-tolerant	Slow	Dry season deciduous
Hura crepitans	Euphorbiaceae	Light-intermediate	Intermediate	Dry season deciduous
Luehea seemanii	Tiliaceae	Early	Fast	Dry season deciduous
Tabebuia rosea	Bignoniaceae	Shade-tolerant	Slow	Dry season deciduous

Notes: Family information from Coll. et al. (2008) and Pérez (2008); successional stage from Scherer-Lorenzen. et al. (2005); approximate relative growth rates from Potvin and Gotelli (2008); leaf phenology from Carrasquilla (2005), Pérez (2008) and Paul et al. (2011).

1.4.2 Experimental design

Tree diversity effects on root decomposition were tested using low and high diversity levels at two spatial scales: meso- (within plot: 2000 m^2) and micro (within bag: 0.02 m^2) (Fig 1.1). At the meso- scale, root decomposition experiments were performed at two sites because they allowed for comparisons of decomposition dynamics under low and high tree overstory diversity levels between strongly contrasting soil conditions (Table 1.2). The low diversity level of tree overstory composition consisted of two single-species plots in Sardinilla of five tree species, *Anacardium excelsum*, *Cedrela odorata*, *Hura crepitans*, *Luehea seemanii*, and *Tabebuia rosea*, and three single-species plots in Agua Salud of two tree species, *Anacardium excelsum* and *Tabebuia rosea*. The high diversity level of tree overstory composition differed at both sites. In Sardinilla, it consisted of three plots that had a five-species mixture in the tree overstory, while in Agua Salud, it

consisted of three plots of secondary forests older than 50 years (Table 1.3). At the micro- scale, the low and high diversity levels refer to the species composition of the coarse roots inside each bag. The low diversity level consisted of single-species coarse roots for each of the five studied tree species, while the high diversity level consisted of a five species coarse root mixture containing all five of the studied species. To avoid confusion, we will use low and high diversity to refer to tree overstory diversity at the meso- scale and single-species and five-species coarse root mixture to refer to low and high root diversity at the micro scale.

In each plot of low tree overstory diversity, five bags with coarse roots matching the composition of the tree overstory and five bags with the five species root mixture were established (summarized in Table 1.3). In total, ten plots with one hundred coarse root bags were used at the low overstory diversity in Sardinilla, while in Agua Salud, six plots with sixty decomposition bags were used. For the high overstory diversity levels in both Sardinilla and Agua Salud, 30 decomposition bags were established per plot (three plots per site), including five single-species bags per species and five bags with a five-species root mixture (90 bags per site). This design allowed us to assess both direct and indirect effects of tree diversity on root decomposition, as well as any potential net diversity effects associated with root diversity inside the bag (single-species vs five species root mixture) under heterogeneous biotic and abiotic conditions. The influence of tree overstory on net diversity effects was tested in two ways using the five species root mixture (Table 1.4). First, this mixture was established in low and high diversity plots in Sardinilla and high diversity plots in Agua Salud to evaluate if net diversity effects due to root interactions inside the decomposition bag were affected by tree overstory composition (Wardle et al. 1997, Loreau 1998). Secondly, single-species and five root mixtures bags were established in plots with the same overstory composition (i.e. *Tabebuia rosea* roots in a *Tabebuia rosea* plots) to calculate the net diversity effect integrating the effect of tree overstory composition with that of root interactions inside the bags.

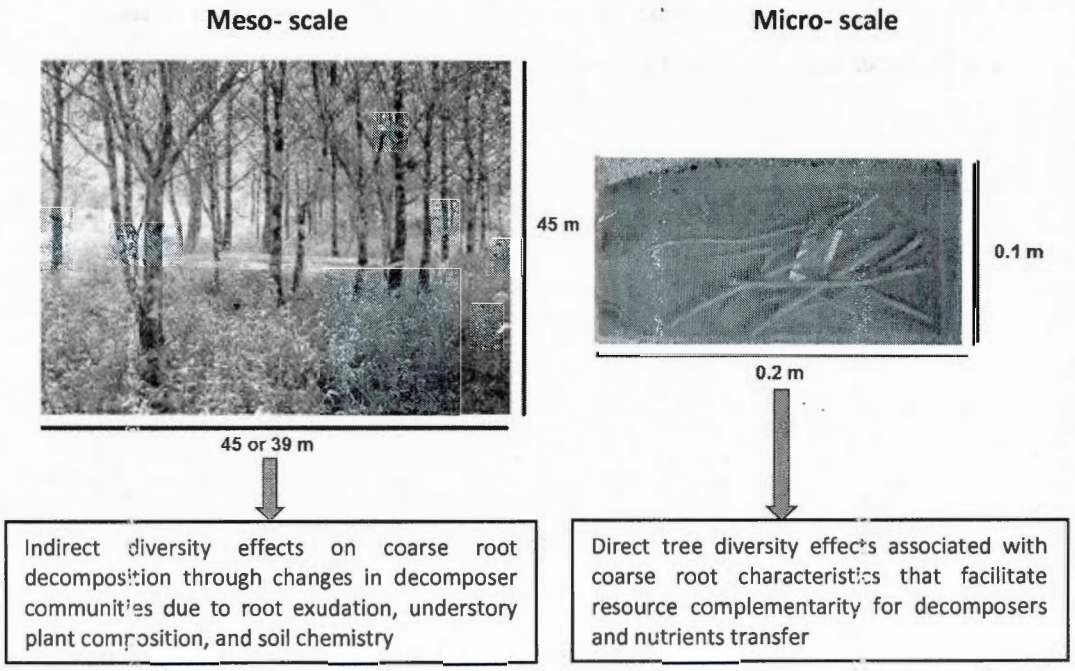

Figure 1.1. Description of the two spatial scales used in our study.

Table 1.2 Soil characteristics and enzymatic soil activity measured in tree diversity plots at the Sardinilla and Agua Salud sites.

Soil variable Mean ± SE	Tree overstory diversity			
	Sardinilla		Agua Salud	
	Low	High	Low	High
Humidity (%)	44.4 ± 2.3	43.5 ± 1.7	45.5 ± 2.8	46.0 ± 3.8
pH (H_20)	5.7 ± 0.04	5.4 ± 0.03	5.5 ± 0.07	-
Carbon (%)	5.5 ± 0.2	6.2 ± 0.7	3.1 ± 0.2	3.5 ± 0.2
Nitrogen (%)	0.5 ± 0.01	0.6 ± 0.06	0.3 ± 0.01	0.3 ± 0.03
Extractable phosphorus (mg kg^{-1}) †	-	3.53 ± 0.38	1.1 ± 0.05	1.5 ± 0.1
Potassium (mmol$_c$ kg^{-1}) †	-	6.5 ± 0.3	-	3.12 ±0.7
Calcium (mmol$_c$ kg^{-1}) †	-	290 ± 8.1	-	70.2 ± 20.4
Magnesium (mmol$_c$ kg^{-1}) †	-	32 ± 1.0	-	37.6 ± 6.8
Aluminum (mmol$_c$ kg^{-1}) †	-	4.0 ±0.1	-	73.7 ± 10.0
ß-Glucosidase (ßG) ‡	8.0 ± 1.0	7.3 ± 0.5	2.9 ± 0.5	1.5 ± 0.1
Cellobiohydrolase (CEL) ‡	1.5 ± 0.2	1.6 ± 0.2	0.6 ± 0.2	0.3 ± 0.04
ß-Xylanase (XYL) ‡	2.3 ± 0.2	2.4 ± 0.3	1.3 ± 0.2	0.8 ± 0.1
N-acetyl-ß-D-glucosaminidase (NAG) ‡	7.5 ± 1.2	8.5 ± 0.6	3.2 ± 0.4	2.5 ± 0.4
Phosphomonoesterase (PME) ‡	28.1 ± 1.9	29.0 ± 3.8	64.5 ± 7.2	95.4 ± 15.1

Notes: †values including the entire plantation in Sardinilla (low and high diversity), ‡values reported in µmol MU g dry min^{-1}. Sardinilla: total carbon, nitrogen and pH from Healy. et al. (2008); extractable phosphorus from Zeugin. et al. (2010), potassium, calcium, magnesium and aluminum from Oelmann et al. (2010), and clay loam texture (Abraham 2004). Agua Salud: pH, total carbon, nitrogen, phosphorus, potassium, calcium, magnesium and aluminum personal communication (Hall and Breugel 2012). Soil texture in Agua Salud varied between silty clay to clay from Hassler et al. (2011).

Table 1.3 Experimental design used to establish the root decomposition experiment at Sardinilla and Agua Salud sites. Number of bags (low and high coarse root diversity levels) established under low or high tree overstory diversity plots are shown. In total, 100 and 60 bags were installed in low tree overstory diversity plots in Sardinilla and Agua Salud respectively. In high tree overstory diversity plots, 90 bags were installed in each study site.

Micro- scale (Coarse root composition)		**Meso- scale (Tree overstory)**								
		Sardinilla						Agua Salud		
		Low diversity (2 plots per species)					High (3 plots)	Low (3 plots per species)		High (3 plots)
		Anacardium excelsum	*Cedrela odorata*	*Hura crepitans*	*Luehea seemanii*	*Tabebuia rosea*	Five species mixture†	*Anacardium excelsum*	*Tabebuia rosea*	Secondary Forest‡
Low	*Anacardium excelsum*	5					5	5		5
	Cedrela odorata		5				5			5
	Hura crepitans			5			5			5
	Luehea seemanii				5		5			5
	Tabebuia Rosea					5	5		5	5
High	Five species mixture	5	5	5	5	5	5	5	5	5

Notes: † include: *Anacardium excelsum, Cedrela odorata, Hura crepitans, Luehea seemanii* and *Tabebuia rosea.* ‡ Old Secondary forest (>50 years) with species richness of 155 spp. 0.2 ha $^{-1}$, personal communication Breugel (2012).

Table 1.4 Composition of roots in decomposition bags in each tree overstory for testing net diversity effects.

Diversity level	Composition of coarse root inside the bags per tree overstory diversity		
	Sardinilla		Agua Salud
	Low diversity	High diversity	High diversity
Root diversity inside decomposition bag across tree overstories	Singles species† and five species mixture	Singles species† and five species mixture	Singles species† and five species mixture
Tree overstory diversity and root diversity inside decomposition bag	Singles species†	Five species root mixture	

Notes: † include: *Anacardium excelsum, Cedrela odorata, Hura crepitans, Luehea seemanii* and *Tabebuia rosea*

1.4.3 Root selection and installation

Coarse root material was excavated in non-experimental plots of all five tree species in the Sardinilla plantation (Table 1.1). Soil particles were removed while leaving the roots intact to determine root orders for each species. Root order was determined for each species based on the classification proposed by Pregitzer et al. (2002) and Valenzuela-Estrada et al. (2008). A digital caliper was used to measure the diameter of each root order to the nearest 0.01± mm. Root order determination was done on two root branches per tree and for ten trees per species. These same trees were subsequently used to collect roots for decomposition experiments. Roots were removed from the soil, washed, and air dried at 40 °C for four days. Roots whose function was to transport and store nutrients were selected (4^{th} and 5^{th} order) and cut to 10 cm in length. Decomposition bags (10 cm x 20 cm) were made using a 2 mm nylon mesh to permit entry of meso-fauna (Fig 1.2a). A total of 5 g dry weight (40 °C) of material was placed carefully in the bags and closed by a manual heat sealer. Aluminum identification tags were placed inside and outside the decomposition bag. For species mixtures, equal proportions of root litter from each species were used. The decomposition bags were established in a 3 m x 3 m space inside the plot avoiding canopy gaps and areas close to

the border. Understory vegetation was cut and removed before installation. Decomposition bags were installed randomly at a depth of 20 cm in a diagonal position, 40 cm apart (Fig 1.2b) using a wooden trowel.

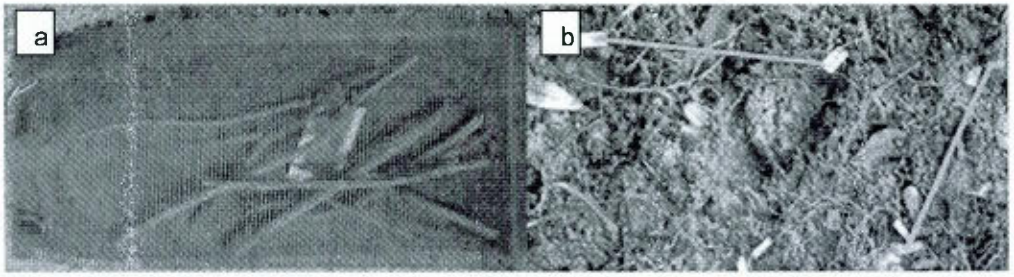

Figure 1.2 Decomposition bag and installation. a) Decomposition bag of 20 x 10 cm with 5 grams of root material and b) bags were separately for 40 cm each.

1.4.4 Root harvest and measurements

Root bags were established in August/September 2011 and collected four times at 50, 160/195 (Agua Salud/Sardinilla), 310 and 485 days. Mass loss at each collection was determined by washing roots carefully over a 2 mm and 250 µm mesh sieve in the first and last two harvests respectively and oven-drying samples at 65 °C for four days. Initial dry weight values at 40 °C were converted to dry mass at 65 °C based on conversion factors calculated for each species (n=5 per species).

1.4.5 Enzymatic Activity after 485 days

Sample from low and high diversity plots in Sardinilla and in Agua Salud were used to measure enzymatic activity on decomposed root after 485 days. Enzymatic activity of five enzymes involved in carbon, e.g. ß-glucosidase (ßG), cellobiohydrolase (CEL) and, ß-xylanase (XYL), nitrogen, e.g. *N*-acetyl-ß-glucosaminidase (NAG) and phosphorus, e.g. phosphomonoesterase (PME), degradation were measured. Characterization of these hydrolytic enzymes was performed with a modified fluorogenic substrate method (Turner and Romero 2010). Complete details about the protocol are presented in Chapter 2.

1.4.6 Root functional traits measurements

A set of root characteristics possibly related to coarse root decomposition were measured. Functional trait measurements included structural, anatomical, and chemical characteristics and were performed on the 4th and 5th root orders for each of the five species. Root diameter, specific root length (SRL), root density, and root dry matter content (RDMC) were measured with a segment of fresh root material (n=5 trees). For each replicate, four measurements on independent roots were taken. Root diameter was measured three times at different points using a caliper. SRL is the relationship between root length and dry weight (cm g^{-1}). Root length was measured with ImageJ (Abramoff et al. 2004) using scanned digital images, while root weight was determined after being oven dried at 65 °C for 48 h. Root volume was measured using the water-displacement method (Chave et al. 2006). Root density was calculated by dividing the volume by the 65 °C oven dried weight. RDMC was measured using a modified method from leaf dry matter content (LDMC) using partial rehydration (Vaieretti et al. 2007).

Anatomical measurements were made using fresh material collected from three different trees in the field and transported to the laboratory immediately. Samples were cut manually, slides were mounted, and images were taken by an Olympus FV1000 confocal microscope using auto-fluorescence. The images were analyzed using ImageJ (Abramoff et al. 2004) to determine: the percent area of xylem, cortex or parenchyma, epidermis (including peridermis), and proportion of vessels in the xylem (Hummel et al. 2007, Chave et al. 2009, Poorter et al. 2010). Although studies about fine root decomposition have not included anatomical measurements frequently, coarse root decomposition could be associated with traits that are correlated with wood decomposition rates (Cornwell et al. 2009, Weedon et al. 2009, Geffen et al. 2010).

For root chemical traits, carbon (C) and nitrogen (N) concentration were determined with an elemental analyzer using five replicates per species (Withington. et al.

2006, Goehel et al. 2011). For determining phosphorus (P) and micronutrient contents, 200 mg of oven-dried ground root material were used; five replicates were analyzed for each species. The sample was suspended in 2 mL of concentrated nitric acid and headed at 180 °C for 6 h. Solutions were measured with an inductively coupled plasma-atomic emission spectrometry (ICP - OES). The micronutrients included aluminum (Al), boron (B), calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), and zinc (Zn). Water-soluble compounds, hemicelluloses and lignin (van Soest method) were quantified using a fiber analyzer system for four trees samples per species (ANKOM Technology, NY, USA). The samples were enclosed in a bag and treated with a series of aggressive extractants to determine 1) neutral detergent fiber percent (% NDF- total fiber), 2) acid detergent fiber percent (% ADF – cellulose, lignin, insoluble ash) and lignin and insoluble ash percent (Alvarez-Clare and Kitajima 2007).

1.4.7 Analysis

1.4.7.1 Tree diversity effects on root decomposition

Two response variables, rates and enzymatic activity after 485 days of decomposition, were used to evaluate tree diversity effects on root decomposition. Decomposition rates (*k*) were calculated using a first order, exponential decay model proposed by Olson (1963). Effects of tree diversity at micro- and meso- scales on root decomposition rates (k $year^{-1}$) and enzymatic activities were analyzed using a linear mixed effect model with the REML estimation method (Zuur et al. 2009) in the in 'lme4' package (Bates and Maechler 2009). First, the most parsimonious random effects structure was determined using Akaike's information criterion (AICc), where site and species identity of the coarse root inside each bag (including the five-species coarse root mixture) were treated as nested, random effects. These effects were selected because the relationship between diversity and root decomposition could be influenced by heterogeneity in abiotic and biotic factors, e.g. soil fertility and root characteristics. The most parsimonious fixed effects structure was subsequently determined where site, tree overstory diversity at the plot level, tree diversity at the bag level, and their interactions

were included as fixed effects in the initial full model. The most parsimonious models were selected using parametric bootstraps and maximum likelihood estimation (Zuur et al. 2009), excluding the fixed factors and interactions that did not contribute information to the model ($p<0.05$). Final models were fit using REML.

To determine the influence of tree overstory and root diversity inside the bag on root decomposition, net diversity effects were estimated using samples from low and high diversity plots in Sardinilla and high diversity plot in Agua Salud. First, proportional deviance was calculated (Wardle et al. 1997, Loreau 1998) from single-species and mixtures using the following equation:

$$Dt = \frac{(Ot - Et)}{Et} * 100$$

where D_t is the proportional deviance of decomposition rates of the root mixture, O_t is the observed decomposition rates taken from the root mixture, and E_t is the expected value of decomposition rates of the root mixture, estimated from the sum of decomposition rates of individual species (Wardle et al. 1997, Loreau 1998). D_t was calculated for the low and high diversity levels in Sardinilla and the high diversity level in Agua Salud. To evaluate the tree diversity effect at different ecological scales, i.e. where overstory tree diversity matches that of the root bag content, D_t was calculated by estimating O_t from the five species mixture in the high diversity level in Sardinilla and E_t from the sum of individual species in the corresponding low diversity level in Sardinilla. Secondly, to test whether D_t reflects non-additive effects, 95 % confidence intervals were calculated using 10,000 bootstrap replicates. If the 95 % CI did not include zero, this would indicate non-additive effects.

1.4.7.2 Functional species identity effects on decomposition

Variation among species based on their root functional traits was visualized using a principal component analysis (PCA) in the vegan package (Oksanen et al. 2013). Due to the number of root functional traits measured and possible multi-collinearity, a correlation analysis was performed. Partial least squares regression (PLSR) was used to determine 1) which root functional traits explain decomposition and 2) if the effects of these functional traits are consistent across sites. PLSR is robust to multi-collinearity among predictor variables and allows predictions when the number of predictor variables exceeds that of observations (Mevik et al. 2011). In the final models, some traits were excluded because of their functional similarity with other traits (based on the PCA). Ratios of C, N, and P were included due to their stoichiometric importance in the decomposition process (Manzoni et al. 2010). PLSR models using root functional traits at each site were calculated using the pls package (Mevik et al. 2011). To evaluate model fit, cross validation was used. The best model was selected based on low values of mean squared errors of prediction (MSEP) and root mean square errors (RMSE). All statistical analyses were performed using R 2.15.3 (R Development Core Team. 2011).

1.5 Results

1.5.1 Root decomposition rates

Variation in root decomposition rates was observed between sites and across species (Table 1.5). Decomposition rates in Sardinilla had lower variation (0.4 – 1.1 g $year^{-1}$) compared with Agua Salud (0.5 – 2.6 g $year^{-1}$). In Agua Salud, on average, decomposition rates were 40% faster than in Sardinilla. The species with the highest k was *Hura crepitans* in both sites with 1.1 and 2.6 g $year^{-1}$ in Sardinilla and in Agua Salud respectively. The species with the lowest decomposition rates varied between sites with *Cedrela odorata* (0.4 g $year^{-1}$) and *Luehea seemanii* (0.5 g $year^{-1}$) in low and high diversity plots in Sardinilla, respectively and *Tabebuia rosea* (0.5 g $year^{-1}$) in both diversity levels in

Agua Salud. *Tabebuia rosea* was the only species that exhibited faster decomposition rates in Sardinilla than in Agua Salud.

Table 1.5 Annual decomposition rate (g $year^{-1}$) for coarse roots of single species and a five species root mixture at two tree overstory diversity levels at the Sardinilla and Agua Salud sites.

Bag composition	Plot diversity			
k year	Sardinilla		Agua Salud	
Mean ± SD	Low	High	Low	High
Anacardium excelsum	0.8 ± 0.1	0.7 ± 0.1	1.4 ± 0.4	1.1 ± 0.1
Cedrela odorata	0.4 ± 0.04	0.6 ± 0.2	NA	1.0 ± 0.3
Hura crepitans	1.0 ± 0.2	1.1 ± 0.2	NA	2.6 ± 0.5
Luehea seemanii	0.6 ± 0.1	0.5 ± 0.1	NA	0.8 ± 0.1
Tabebuia rosea	0.7 ± 0.1	0.6 ± 0.1	0.5 ± 0.2	0.5 ± 0.1
Five species mixture	0.7 ± 0.1	0.7 ± 0.04	0.9 ± 0.2	1.1 ± 0.1

Notes: Mean values were calculated in Sardinilla for singles species (n=2) and five species root mixtures (n=10) in the low diversity level, and for all bag compositions (n=3) in the high diversity level. Mean values were calculated in Agua Salud for all bag compositions (n=3) at both tree diversity levels. NA: Plot not available in Agua Salud.

1.5.2 Tree diversity effects on coarse root decomposition

We found a consistent lack of evidence in support of direct or indirect diversity effects on root decomposition rates and enzymatic activity. While the most parsimonious model explained 80% of decomposition rates (Table 1.6, Table S1.1), this model did not include tree diversity at either the micro and meso- scales. However, a strong site effect on coarse root decomposition rates was present in most models. Similar patterns were observed for enzymatic activity, where only site was included as a fixed effect. However, the final models only explained between 6 to 38 % of the variation found in enzymatic activity, indicating that other factors not tested in the study could influence enzymatic activity during root decomposition.

Table 1 6. Effects of site and two scales of diversity on root decomposition rates and enzymatic activity after 485 days of decomposition.

Response variable	AICc	ΔAICc	R^2 (%)	Factors		Explained variation† (%)
				Fixed	Random	
k year	130.7	2.0	80.0	Site	Species identity	87.0
					Site	10.8
ß-glucosidase	188.0	2.8	27.8	Site	Species identity*	0.7
Cellobiohydrolase	187.2	2.1	27.2	Site	Species identity*	0.3
ß-xylanase	190.6	0.6	37.9	Site*	Species Identity	19.0
					Site	56.6
N-acetyl-ß-D-glucosaminidase	200.3	2.2	6.1	-	Species identity*	0.2
Phosphomonoesterase	189.0	2.6	24.2	Site	Species identity*	0.2

Notes: For each response variable, a mixed effects model was fit with REML after determining the most parsimonious random and fixed effects structure. Tested random effects included species identity of root material in decomposition and site. In the initial, full models, site, diversity at micro and meso- scales, and their interactions were included as fixed effects. † Variation explained by random effects.

1.5.3 Net diversity effects on coarse root decomposition

The influence of tree overstory composition on net diversity effects measured using five species root mixture showed contrasting results. No significant net diversity effects (additive effects) in *k* values were observed in the five species root mixture across the low and high diversity levels in Sardinilla and high diversity level in Agua Salud (Fig 1.5, white boxes). As well, no net diversity effects in *k* values were found when observed and expected values from the high and low tree diversity levels in Sardinilla were compared (Fig 1.3, gray box). However, for enzymatic activity, although no net diversity effect was found in high tree overstory diversity levels in Sardinilla and in Agua Salud, a negative net diversity effect (non-additive) was observed in the low diversity level in Sardinilla (Fig 1.4, white boxes). Negative effects also were found in high and

low tree diversity levels in Sardinilla for almost every enzymatic activity, with the exception of NAG (Fig 1.4, white boxes).

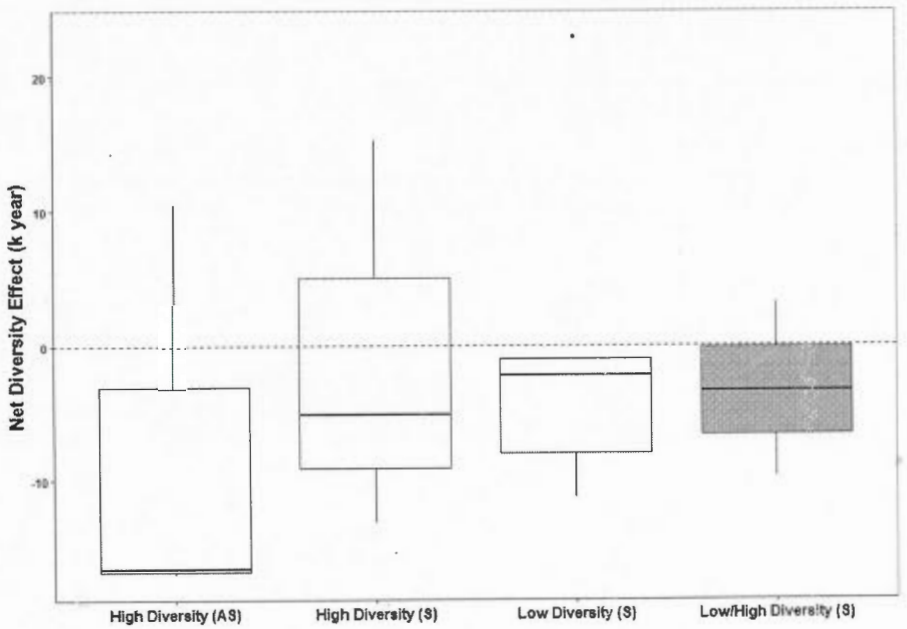

Figure 1.3 Net diversity effects in five root species mixtures using the annual *k*. White boxes represent five species root mixture across contrasting tree overstory diversities. The gray box represents the comparison between single species in low diversity plots and the five species root mixture installed in the high diversity plots in Sardinilla. Agua Salud (AS) and Sardinilla (S).

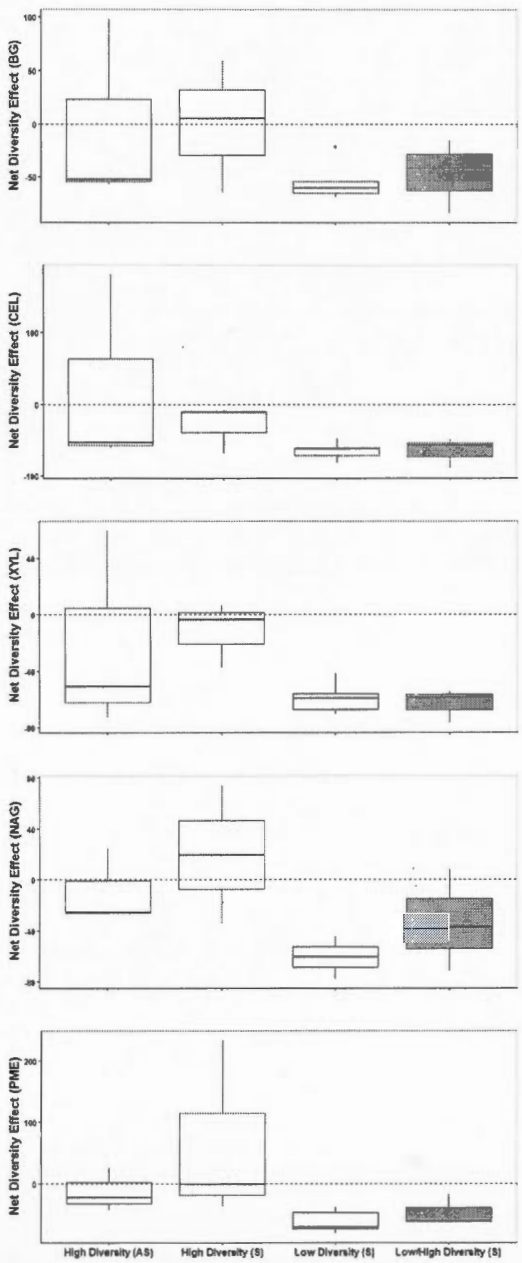

Figure 1.4 Net diversity effects in five root species mixtures for enzymatic activity after 485 days. White boxes represent five species root mixture across contrasting tree overstory composition. The gray box represents the comparison in Sardinilla betwen single species samples in low diversity plots and the five species root mixture samples installed in the high diversity plots.

1.5.4 Functional species root identity effects on decomposition

PCA analysis revealed that the studied species differentiated markedly in terms of function (Fig 1.5, Table S1.2). The first two axes of the principal component analysis using root traits explained 80 % of the variation observed across five species. *Anacardium excelsum, Hura crepitans,* and *Tabebuia rosea* were strongly associated with the first axis (PC1) while *Cedrela odorata* and *Luehea seemanii* with the second axis (PC2). PC1 was comprised principally of chemical traits, as well as some structural (SRL, and diameter) and anatomical (epidermis and xylem) traits. PC2 was defined principally by structural traits. The contribution of chemical and anatomical traits to PC2 was limited to Zn and Mg content and xylem area. The water soluble fraction of tissue was an important trait in both axes. Correlation analysis exhibited similar relationships among root functional traits (Table S1.3).

Despite PLSR models showing that root functional traits predicted root decomposition rates in both sites, the explained proportion of root decomposition using root traits varied between sites. PLSR model using data from both sites explained 35 % of variation in k, separate models for Sardinilla and Agua Salud explained 37 % and 80 % of this variation, respectively (Table 1.6). Also, in spite of some root functional traits significantly explaining root decomposition rates in all models, the magnitude of the size effect varied for traits between sites (Fig 1.6, Table S1.4). In the PLSR model using data from both sites, traits that explained root decomposition significantly ($p<0.05$) were Al, ash, C:P, lignin, Mn, RDMC, and vessel area proportion. The PLSR model for Agua Salud also included C:P, Ca, cellulose, and cortex area proportion as significant variables. Root Na content was significant in Sardinilla and Agua Salud models separately, but not when analyzed together. As a result of PLSR models and the different responses of the species to site conditions (reflected in their root decomposition rates), the interaction between root functional traits and sites were analyzed (Fig 1.7). For instance, a functional trait related to C quality, i.e. lignin, explained 61 % of the site differences. In other words, while less recalcitrant root material decomposed up to ~60 % faster in

Agua Salud than in Sardinilla, more recalcitrant root initial material decomposed ~30 % faster in Sardinilla than in Agua Salud.

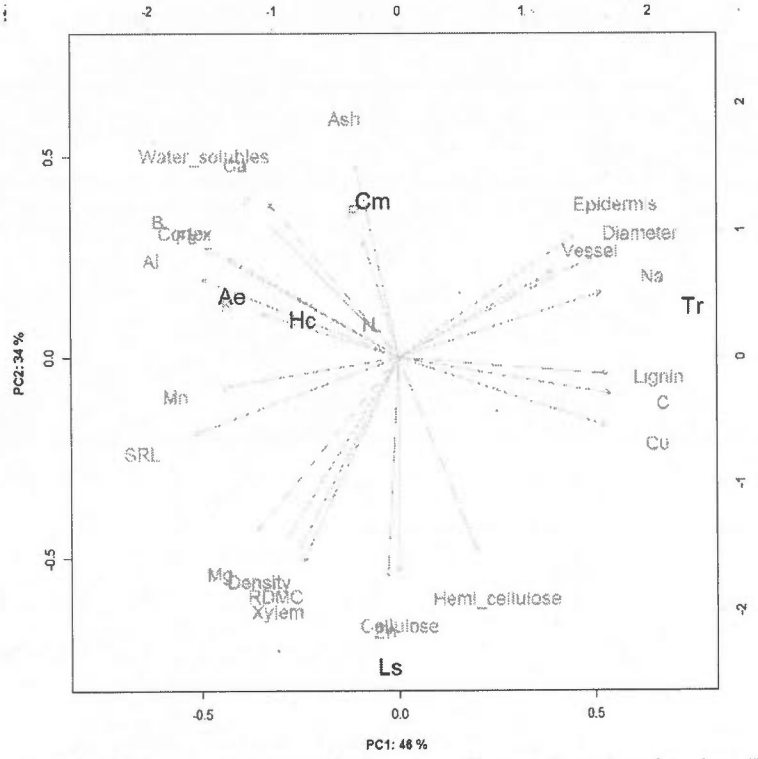

Figure 1.5 Principal component analysis using all root functional traits. Five species used: Ae: *Anacardium excelsum,* Cm: *Cedrela odorata,* Hc: *Hura crepitans,* Ls: *Luehea seemanii* and Tr: *Tabebuia rosea.* Root functional traits included: SRL: specific root length, RDCM: Root dry matter content, Vessel: vessel proportion related to xylem area, C: carbon, N: nitrogen, P: phosphorus, Al: aluminium, B: boron, Ca: calcium, Cu: copper, Fe: iron, K: potassium, Mg: magnesium, Mn: manganese, Na: sodium and, Zn: zinc.

Table 1.7 Partial least squares regressions (PLSR) using root functional traits to explain decomposition rates.

Variable	Site	Component number	MSEP	RMSEP	R^2
	Sardinilla	3	0.59	0.77	37.1
k constant	Agua Salud	3	0.19	0.44	79.6
	Both	3	0.63	0.80	34.6

Abbreviations: MSEP: Mean squared error of prediction and RMSE: Root mean square error. R^2 values show the percent of variation explained by the model.

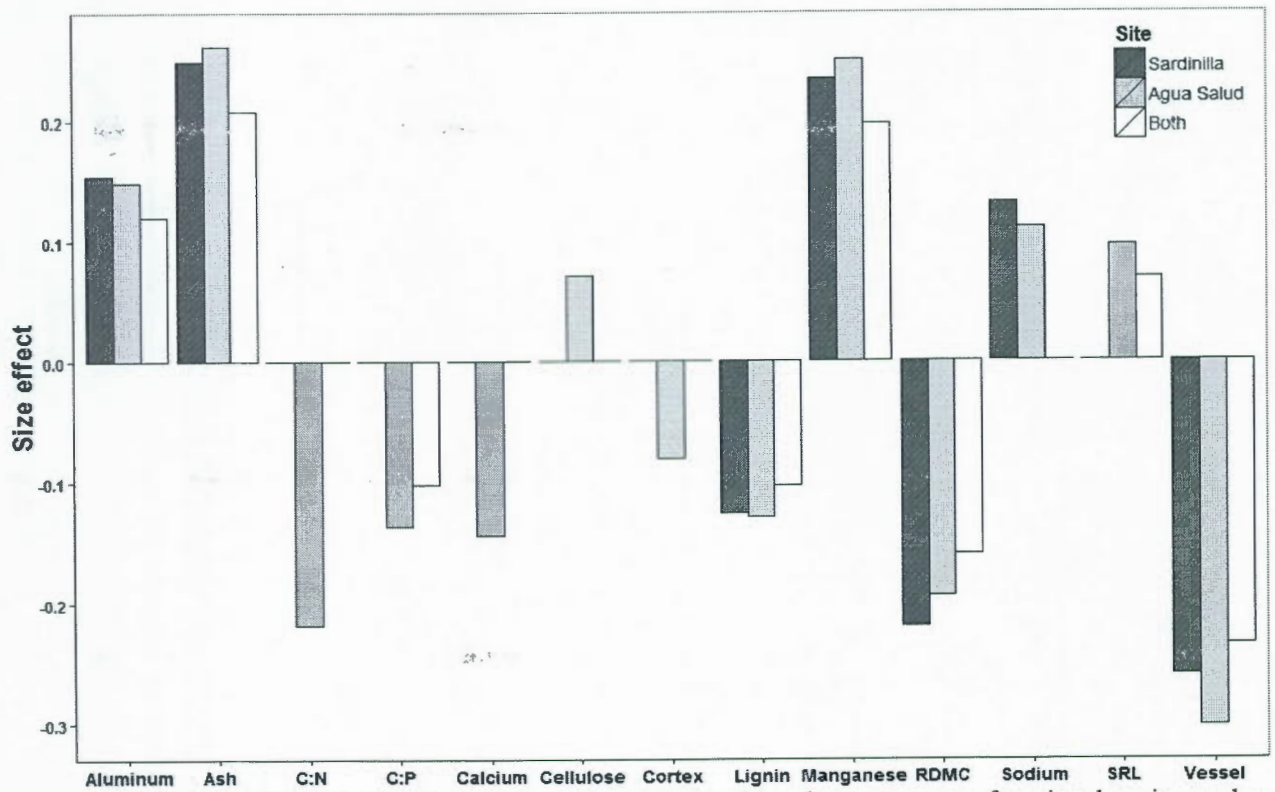

Figure 1.6 Coefficients of fitted PLSR models testing relationships between root functional traits and root litter decomposition. Abbreviations: RDMC: root dry matter content and SRL: specific root length.

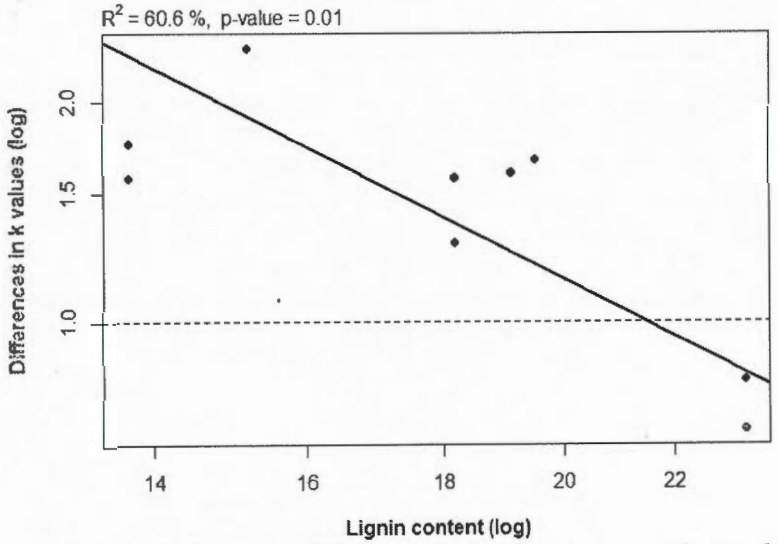

Figure. 1.7 Correlation between differences in *k* values between sites and initial root lignin content. Values higher than 1 indicate faster decomposition in Agua Salud than in Sardinilla while values lower than 1 indicate faster decomposition in Sardinilla than in Agua Salud. Differences in *k* values were calculated by dividing the values of decomposition from both sites for each species.

1.6 Discussion

Contrary to our expectations, tree diversity did not influence root decomposition neither at meso- scale nor at the micro- scale across sites. Patterns of decomposition were strongly modulated by functional identity, yet these responses were also inconsistent across study sites. Our results suggest that in tropical forests, tree communities directly influence root decomposition mainly based on functional identity. However, the high variance associated with the calculated mean net diversity effects in five species root mixtures (Fig. 1.3) suggest that the influence of external factors, such as spatial heterogeneity of abiotic and biotic conditions, likely overshadowed diversity effects at micro- scale. Herein, we will discuss the ecological implications of our results.

1.6.1 Tree diversity effects on coarse root decomposition

In the present study, tree overstory diversity did not influence coarse root decomposition rates or enzymatic activity across sites. Our results concur with those found by Scherer-Lorenzen et al. (2007), where the indirect influence of tree overstory diversity on decomposition was not found in a leaf decomposition using the same five species in Sardinilla. While diversity effects on decomposition, mainly using leaf litter, have been found in other studies, these effects have been either weak (Handa et al. submitted) or less consistent than effects on other, well-described ecosystem functions such as productivity (Cardinale et al. 2011, Hooper et al. 2012).

Tree overstory diversity effects on root decomposition at the meso-scale could be mitigated by intrinsic environmental conditions associated with this process. Soil could provide a buffer for indirect tree overstory diversity effects on micro-climatic conditions (Silver and Miya 2001). As well, soil properties and environmental factors could influence micro- and meso-decomposer communities more strongly than tree diversity in our study. For example, studies have shown that fungal:bacterial ratios depend in great part to the C:N ratio of the soil (Fierer et al. 2009). Factors like soil texture and seasonality have also been shown to influence root decomposition in tropical grasses (Gijsman et al. 1997) by affecting the activity and mobility of decomposers. In our study, we assumed that the understory vegetation was influenced, in terms of composition, by tree overstory identity and diversity (Li et al. 2012). Still, a considerable portion of root biomass from the understory vegetation is located in the first soil layers, i.e. where the root bags were installed. Consequently, understory vegetation could influence tree root decomposition directly or indirectly by influencing soil communities, e.g. fungal and bacterial compositions (Wardle and Zackrisson 2005, Wu et al. 2011) and providing additional litter with contrasting quality to the system (Zhao et al. 2013). Still, the relationship between tree root decomposition and understory vegetation needs to be tested. While tree diversity effects were not observed in our study, direct effects of tree overstory composition may indeed influence

belowground processes through specific tree identity effects that may be associated with litter traits (Thoms et al. 2010).

The absence of diversity effects on decomposition in the present study also could be related to temporal and spatial scales. Despite using one of the oldest experimental biodiversity plantations established in Latin America and an old secondary forest (10 years in Sardinilla and >50 years in Agua Salud), these temporal scales could be too brief for diversity effects to emerge. Recently, diversity effects on ecosystem processes have been found to increase with time (Cardinale et al. 2011, Allan et al. 2013). However, most studies using temporal scales have been performed in grasslands (e.g. Isbell et al. 2011, Reich et al. 2012), which have much faster species turnover rates than forests (Gill and Jackson 2000). Hence, biotic interactions leading to density-dependent mortality or "home field advantage" (Ayres et al. 2009) might become apparent much later in tree communities compared to grasslands.

At two distinct spatial scales, significant effects of diversity on root decomposition were not found. Contrary to our hypothesis of complementary resource availability at the micro scale (Hättenschwiler et al. 2005), direct effects of tree diversity were not consistently observed. Indirect effects of tree overstory composition were not reflected in decomposition rates at the meso- scale either. While this study is one of the first studies to compare tree root decomposition rates between two levels of tree diversity using experimental plots, we suggest using a more flexible spatial approach to detect diversity effects, such as the neighborhood analysis used Potvin and Dutilleul (2009).

1.6.2 Net diversity effects on coarse root decomposition

No significant net diversity effects (NDE) at the micro scale (Fig. 1.3) reflected the greater relative importance of species identity in determining root decomposition rates relative to species interactions. The high explanatory power of both species identity and functional traits in predicting decomposition rates provide further evidence

in support of our finding of additive effects of species at the micro scale. The high variation observed in NDE across plots (-13 to 15% and -17 to 10% in high diversity plots in Sardinilla and Agua Salud, respectively), indicates that environmental heterogeneity also strongly influenced decomposition at the micro scale. Similarly, NDE on enzymatic activity were highly variable, as both additive and non-additive effects were observed for the same five species root mixture. While few studies using leaf mixtures in multiple sites have been performed, across-site variation has been observed frequently and has been attributed to heterogeneity of abiotic and biotic conditions (e.g. Madritch and Cardinale 2007, Jonsson and Wardle 2008, Hoorens et al. 2012).

Although decomposition rates and enzymatic activity are both indicators of the process of litter degradation, they reflect different aspects of the same process: decomposition rates reflect degradation over the entire process and enzymatic activity indicates temporally dynamic shifts in degradation. Perhaps due to these differences, we observed contrasting NDE between decomposition rates and enzymatic activity at the micro scale in the present study. Decomposition is a dynamic process where fluctuations in litter quality, succession of decomposer communities, and micro-site conditions could result in different interactions at the micro scale (within the decomposition bag) that would alter the direction and magnitude of diversity effects (Gartner and Cardon 2004). From a biochemical perspective, negative non-additive values observed in the present study could be explained by substrate diversity, as organic N and P could be obtained from less recalcitrant substrates, thereby decreasing investment in *N*-acetyl-ß-D-glucosaminidase and phosphomonoesterase, respectively (Sinsabaugh and Moorhead 1994). Also, due to the diversity of substrate C quality, extracellular enzymes could be acting on multiple substrates simultaneously.

1.6.3 Effects of functional composition on coarse root decomposition

The observed patterns of interspecific variation among an extensive suite of root functional traits (27 traits) provide novel insight to relationships among anatomical, structural, and chemical root traits for tropical tree species. Previous studies in tropical

ecosystems have emphasized leaf and stem functional traits (e.g. Kraft et al. 2008, Swenson and Enquist 2009, Uriarte et al. 2010, Wright et al. 2010), despite the increasing appreciation of the importance of belowground processes in tropical forests (Mangan et al. 2010). Even when belowground processes have been explicitly included, the limited number of belowground traits measured has prevented the generalization of a trait syndrome for roots similar to that of leaf (Wright et al. 2004) and stem traits (Chave et al. 2009). For example, a study on the functional strategies of 758 Neotropical tree species, Fortunel et al. (2012), included only one root functional trait (root wood density), yet 13 leaf functional traits. Our results provide a basis for selecting informative root functional traits, thus enabling a larger number of species to be measured in future studies.

Our study illustrates the importance of root traits as a predictor of coarse root decomposition in tropical trees. Root functional traits explained 37and 80% of variation in decomposition in Sardinilla and Agua Salud, respectively. Across sites, faster decomposition was associated with high values of SRL and ash, Al, and Mn content, and low values of RMDC, vessel area proportion, lignin content, and C:P. The influence of leaf and root traits associated with C quality, such as lignin, on decomposition has been reported elsewhere (Hättenschwiler et al. 2011, Freschet et al. 2012). Mn content has been found to exert significant, positive effects on decomposition as it is a component of Mn-peroxidase, an enzyme involved in lignin degradation (Baldrian and Snajdr 2011). Vessel area proportion was strongly and negatively associated with coarse root decomposition in our study and has been found to be a strong predictor of wood decomposition (Cornwell et al. 2009, Geffen et al. 2010). Larger vessel area proportion could be linked to slow decomposition because it reduces access to microorganisms when interstitial spaces are plugged with tyloses, gum, or resin (Geffen et al. 2010). Finally, the significantly negative effects of root C:P could be related to the importance of high P content in roots in P poor soil, which are as old tropical soils (Vitousek and Sanford 1986). Also, P limitation in soils on root decomposition was suggested due to

the high quantities of phosphomonoesterase produced during root decomposition (see Chapter 2).

The importance of sites effects was reflected in the contrasting root decomposition rates found in Sardinilla and in Agua Salud. Similar to other ecosystem processes, abiotic resource heterogeneity could influence diversity effects (Tylianakis et al. 2008, Duffy 2009, Cardinale et al. 2012). In our study, the greatest differences in abiotic conditions between study sites are likely related to soil properties. Despite our hypothesis about soil properties being an important driver of root decomposition, it is necessary to interpret these results with caution, as it is necessary to separate the influence of soil properties from other factors, like tree overstory diversity. Soil fertility could influence decomposer communities, as lower fertility soils are associated with fungus, while more fertile soils are associated with bacteria (Wardle et al. 2004). Thus, the fast decomposition observed at Agua Salud is likely due, in part, to its lower soil fertility, as fungi are more efficient at decomposing C than bacteria (Rhee et al. 1987). However, studies linking soil decomposer communities are less frequent in the tropics, where fungal community composition was not related either to soil nutrient and tree diversity in a leaf decomposition study (McGuire et al. 2011). Differences in topography between Sardinilla (flat landscape) and Agua Salud (steep slopes) could have influenced hydrological processes (Hassler et al. 2011, Wohl et al. 2012), generating either oxic or anoxic conditions, which could modulate decomposition rates (Lee 1992). Finally, it is important to highlight that species did not respond uniformly to site conditions. The most recalcitrant species based on lignin content, *Tabebuia rosea*, decomposed faster in Sardinilla, while the least recalcitrant species, *Hura crepitans* and *Anacardium excelsum*, decomposed fastest in Agua Salud. These results suggest that root decomposition rates are jointly determined by functional identity and site conditions.

1.7 Conclusions

Our results show that tropical tree communities did not influence root decomposition through their overstory diversity at two distinct spatial scales, but rather through functional identity. As a result, changes in tree overstory composition in tropical regions that alter functional composition, such as land-use change or drought (Phillips et al. 2010), could have significant repercussions on ecosystem processes such as root decomposition. Secondly, we emphasize the importance of considering multiple scales and multiple sites for exploring tree diversity effects on decomposition, as well as on other belowground ecosystems processes (Madritch and Cardinale 2007). Finally, soil decomposer communities that are linked to intrinsic soil characteristics may also strongly modulate root decomposition.

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1.9 Supplementary material

Table S1.1 Mixed effects model for explaining root decomposition rates and enzymatic activity after 485 days.

Model	AICc					
	k year	ßG	CEL	XYL	NAG	PME
Three-way interaction + two-way interactions + single factors	146.1	200.8	198.3	202.2	213.2	201.1
Two-way interactions + single factors	144.8	200.1	197.5	201.3	213.3	200.8
Two-way interactions (except meso- scale diversity x micro scale diversity) + single factors	142.0	198.1	195.3	198.9	212.0	199.4
Two-way interactions (except site x micro- scale diversity) + single factors	143.7	198.0	196.5	200.6	211.5	198.7
Two-way interactions (except site x meso- scale diversity) + single factors	141.2	197.8	195.2	199.7	211.6	198.6
Single factors	137.4	193.7	192.2	196.8	208.7	195.1
Site + meso- scale diversity	135.3	191.4	190.4	194.4	206.2	192.5
Site + micro- scale diversity	132.7	190.8	189.3	193.7	205.9	191.6
Meso- scale + micro scale diversity	142.0	205.8	204.9	196.7	205.3	206.8
Site	130.7	188.0	187.2	190.6	203.6	189.0
Meso- scale diversity	139.9	203.6	203.2	194.4	202.9	203.8
Micro- scale diversity	137.4	202.7	203.1	193.9	202.5	203.8
Without factors	135.4	200.4	200.7	191.2	200.3	200.7

Notes: ßG: ß-glucosidase; CEL: cellobiohydrolase; XYL: ß-xylanase; NAG: *N*-acetyl-ß-glucosaminidase; and PME: phosphomonoesterase (PME).

Table S1.2 Root functional traits values for five tree species, including: structural, anatomical and chemical traits.

Root traits Mean ± SD	Species				
	Anacardium excelsum	*Cedrela odorata*	*Hura crepitans*	*Luehea seemanii*	*Tabebuia rosea*
Structural traits					
Diameter (cm)	2.6 ± 0.2	2.8 ± 0.1	2.8 ± 0.5	2.3 ± 0.1	4.0 ± 0.7
SRL (cm g^{-1})	65.2 ± 11.8	59.1 ± 8.0	68.3 ± 10.4	67.2 ± 15.0	41.2 ± 12.2
RMDC	0.3 ± 0.02	0.2 ± 0.02	0.2 ± 0.02	0.3 ± 0.01	0.2 ± 0.01
Root density	0.3 ± 0.01	0.2 ± 0.02	0.2 ± 0.02	0.3 ±0.01	0.2 ± 0.002
Water soluble†	47.5 ± 2.2	44.1 ± 3.0	43.0 ± 3.0	34.2 ± 0.9	36.1 ± 2.0
Hemicellulose†	9.8 ± 0.5	10.3 ± 0.2	10.9 ± 0.9	12.1 ± 1.2	11.0 ± 0.4
Cellulose†	28.6 ± 2.3	25.7 ± 2.9	30.6 ± 3.3	34.5 ± 1.8	29.2 ± 1.7
Lignin†	13.7 ± 0.4	19.5 ± 2.4	15.2 ± 1.2	19.1 ± 0.9	23.4 ± 2.2
Ash†	0.3 ± 0.2	0.4 ± 0.1	0.4 ± 0.2	0.2 ± 0.02	0.3 ± 0.1
Anatomical traits					
Epidermis area†	16.3 ± 4.9	20.4 ± 2.6	22.8 ± 2.8	9.8 ± 3.4	38.1 ±6.2
Cortex area†	56.2 ± 4.9	64.2 ± 7.1	49.0 ± 4.2	41.9 ± 3.8	30.4 ± 7.4
Xylem area†	27.6 ± 5.1	15.3 ± 4.7	28.2 ± 3.3	48.3 ± 5.7	12.9 ± 5.8
Vascular area†	27.6 ± 5.1	15.3 ± 4.7	28.2 ± 3.3	48.3 ± 5.7	31.4 ± 5.6
Vessel area†	17.0 ± 3.0	23.2 ± 5.0	10.4 ± 2.5	15.4 ± 2.4	25.1 ± 4.5
Chemical traits					
Carbon‡	446.5 ± 4.1	453.7 ±7.0	441.0 ± 7.3	461.1 ± 3.5	481.4 ± 12.9
Nitrogen‡	5.6 ± 0.6	10.3 ± 2.1	9.8 ± 1.9	8.2 ± 0.7	7.0 ± 0.7
Phosphorus‡	0.7 ± 0.1	3.1 ± 1.5	1.2 ± 0.6	0.7 ± 0.2	0.7 ± 0.1
Aluminum‡	3.5 ± 2.2	2.3 ± 1.1	3.2 ± 2.2	1.7 ± 0.3	1.2 ± 0.5
Boron‡	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.005	0.02 ± 0.002
Calcium‡	21.8 ± 1.9	21.8 ± 2.0	11.9 ± 2.0	6.8 ± 1.9	8.0 ± 1.1
Cupper‡	0.01 ± 0.004	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.004
Iron‡	2.3 ± 1.8	1.2 ± 0.7	1.9 ± 1.3	0.7 ± 0.3	0.8 ± 0.3
Potassium‡	3.7 ± 1.3	8.6 ± 3.1	5.2 ± 1.5	4.5 ± 1.6	0.3 ± 0.2
Magnesium‡	4.4 ± 0.9	2.7 ± 0.8	3.7 ± 0.5	6.2 ± 1.1	1.3 ± 0.2
Manganese‡	0.1 ± 0.07	0.1 ± 0.03	0.1 ± 0.07	0.1 ± 0.09	0.04 ± 0.02
Sodium‡	0.6 ± 0.3	2.1 ± 1.1	2.8 ± 1.1	1.0 ± 0.6	7.7 ± 1.3
Zinc‡	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.3 ± 0.1	0.01 ± 0.01

† Values in percent, in anatomical measurements is the area percent in the cross-section while vessel area was calculated is respect to the xylem, ‡ Values reported in mg g^{-1}.

Table S1.3. Correlation matrix across root functional traits. Significant values in bold ($p<0.05$)

	SRL	Diam	Den	RDMC	Epid	Cort	Xyl	Ves	C	N	P	NPE	WS	AS	Lig	Al	B	Ca	Cu	Fe	K	Mg	Mn
Diam	**-0.95**																						
Den	0.67	-0.82																					
RDMC	0.65	-0.81	**0.99**																				
Epid	**-0.87**	**0.98**	**-0.90**	**-0.89**																			
Cort	0.55	-0.58	0.13	0.10	0.51																		
Xyl	0.71	-0.76	**0.91**	**0.93**	0.80	0.09																	
Ves	-0.84	0.67	-0.43	-0.43	0.55	0.13	0.67																
C	**-0.87**	0.74	-0.26	-0.22	0.60	0.74	0.28	0.74															
N	0.24	-0.19	-0.18	-0.07	0.08	0.35	0.06	0.16	0.29														
P	0.04	-0.11	-0.25	-0.20	0.07	0.72	0.42	0.32	0.24	0.72													
NPE	0.36	-0.26	-0.18	-0.28	0.16	0.80	0.33	0.18	0.73	0.02	0.36												
WS	0.04	-0.10	0.41	0.51	0.16	0.62	0.62	0.20	0.38	0.22	0.33	**0.91**											
AS	0.34	-0.35	0.65	0.69	0.39	0.56	**0.87**	0.55	0.09	0.14	0.68	0.66	0.84										
Lig	-0.82	0.69	-0.35	-0.27	0.58	0.58	0.35	0.75	**0.92**	0.10	0.10	0.73	0.43	0.02									
Al	0.68	-0.53	0.11	0.02	0.40	0.67	0.09	0.62	**0.91**	0.05	0.05	**0.88**	0.64	0.23	**0.96**								
B	0.61	-0.49	0.04	-0.05	0.37	0.78	0.05	0.45	**0.89**	0.00	0.23	**0.95**	0.75	0.42	**0.90**	**0.98**							
Ca	0.23	-0.26	-0.09	-0.17	0.22	**0.89**	0.38	0.13	0.55	0.00	0.58	**0.91**	**0.89**	0.78	0.50	0.66	0.80						
Cu	-0.70	0.64	-0.27	-0.17	0.56	0.80	0.13	0.47	**0.88**	0.09	0.18	**0.89**	0.67	0.29	**0.93**	**0.95**	**0.97**	0.79					
Fe	0.51	-0.35	-0.01	-0.12	0.23	0.54	0.04	0.50	0.78	0.25	0.08	**0.88**	0.73	0.29	**0.91**	**0.97**	**0.94**	0.64	**0.90**				
K	0.58	-0.63	0.20	0.23	0.56	**0.87**	0.07	0.19	0.63	0.72	0.84	0.44	0.20	0.33	0.33	0.37	0.47	0.57	0.49	0.16			
Mg	0.82	-0.89	**0.96**	**0.95**	**0.93**	0.17	**0.96**	0.67	0.46	0.08	0.28	0.09	0.40	0.69	0.53	0.29	0.18	0.10	0.38	0.15	0.25		
Mn	0.80	-0.61	0.34	0.29	0.49	0.27	0.49	**0.92**	0.82	0.09	0.36	0.47	0.15	0.32	**0.92**	0.84	0.70	0.15	0.71	0.79	0.11	0.57	
Na	**-0.91**	**0.98**	-0.77	-0.74	**0.96**	0.71	0.63	0.56	0.76	0.12	0.20	0.42	0.09	0.17	0.73	0.61	0.60	0.45	0.75	0.44	0.67	0.82	0.57

Abbreviations SRL: specific root length, Diam: diameter, Den: density, RDMC: Root dry matter content, Epid: epidermis, Cort: Cortex, Xyl: xylem, Ves: vessel proportion related to xylem area, C: carbon, N: nitrogen, P: phosphorus, NPE: Water soluble, WS: hemicelluloses, AS: cellulose, Lig: lignin, Al: aluminium, B: Boron, Ca: calcium, Cu: cupper, Fe: iron, K: potassium, Mg: magnesium, Mn: manganese and, Na: sodium.

Table S1.4 Coefficient values of partial least square regressions using root functional traits to explain decomposition rates. Only significant coefficients (p-values <0.05) are reported.

Trait	Size effects on decomposition rates		
	Sardinilla	Agua Salud	Both
SRL		0.096	0.069
RMDC	-0.220	-0.194	-0.160
Lignin	-0.126	-0.129	-0.103
Cellulose		0.071	
Ash	0.249	0.261	0.207
Cortex		-0.080	
Vessel	-0.260	-0.302	-0.234
C:N		-0.218	
C:P		-0.137	-0.102
Aluminum	0.154	0.149	0.120
Calcium		-0.144	
Manganese	0.234	0.250	0.197
Sodium	0.132	0.110	

Notes: water soluble, hemicellulose, xylem area, N:P, and K were used but no significant results were found.

CHAPTER II

EXPANDING THE USE OF ENZYMATIC ACTIVITY IN COARSE ROOT DECOMPOSITION STUDIES IN TROPICAL FORESTS

2.1 Résumé

Les communautés microbiennes utilisent un ensemble diversifié d'enzyme pour dégrader le carbone (C) des litières de plantes et acquérir l'azote (N) et le phosphore (P) au cours des différentes étapes du processus de décomposition. En dépit du rôle majeur des enzymes pour la décomposition des litières, les études incluant cette perspective biochimique sont rares et restreintes principalement à une poignée d'études utilisant des litières d'écosystèmes tempérés. Notre principal objectif de ce second chapitre était de développer un protocole standard pour mesurer l'activité enzymatique des décomposeurs dans les études de décomposition des racines et de démontrer son application écologique. Nous suggérons des valeurs de paramètres, notamment la concentration du substrat, le pH optimal, et le temps d'incubation basé sur des essais avec du matériel décomposé de cinq espèces tropicales locales et un mélange de racines de cinq espèces. Les valeurs de l'activité enzymatique associée au C, à l'N et au P ont été utilisées pour évaluer l'importance relative de ces nutriments dans les taux de décomposition au cours du temps à deux endroits caractérisés par des propriétés du sol contrastés au Panama, Sardinilla et Agua Salud. L'expérience de terrain a duré 485 jours et l'activité enzymatique a été mesurée après 50, 310, et 485 jours de décomposition. Les enzymes étudiées liés à la dégradation de C étaient cellobiohydrolase (CEL), ß-glucosidase (ßG), et ß-xylanase (XYL). Les enzymes liés à l'acquisition de N et P étaient *N*-acetyl ß-glucosaminidase (NAG) and phosphomonoesterase (PME). Afin de déterminer les paramètres optimaux pour mesurer l'activité enzymatique lors des essais, les enzymes α-glucosidase (AG) et sulfatase aryle (AS) ont aussi été incluses. Parmi les enzymes, nos résultats indiquent que l'activité enzymatique dans les racines décomposées doit être mesurée en utilisant une concentration en substrat de 100-200 µmol et un tampon de pH 4.8 à 5. Les temps d'incubation varient entre enzymes, de 45 min pour PME à > 180 min pour AG, CEL, XYL et AS. En accord avec notre hypothèse initiale, nous avons observé une relation entre l'activité enzymatique et la décomposition des racines au travers des espèces et des sites d'étude. L'activité enzymatique cumulative prédisait entre 21 et 63% des taux de décomposition. L'activité des enzymes liées à l'acquisition de N et P, en particulier, a été fortement corrélé avec les taux de décomposition, c'est à dire une décomposition plus rapide a été associée avec une plus forte activité enzymatique cumulée. L'activité des enzymes associées au C était quant à elle reliée à l'identité de l'espèce, même si les effets de site étaient également importants, ce qui indique que des facteurs externes, à savoir les différences dans les communautés de décomposeurs ou les propriétés du sol peuvent également moduler les taux de décomposition. De façon inattendue, l'activité enzymatique cumulative des enzymes carbonées n'a pas été expliquée par les caractéristiques des racines qui décrivaient la qualité du C. La variation de l'activité

enzymatique liée à l'N a été principalement expliquée par l'activité enzymatique du sol, alors que la variation de l'activité enzymatique associée au P a été expliqué par les traits fonctionnels des racines. Bien que différents facteurs influencent clairement le rôle de ces enzymes lors de la décomposition, la quantification de l'activité enzymatique nous a permis d'évaluer l'importance relative des différents groupes fonctionnels de décomposeurs microbiens pendant le processus de décomposition racinaire.

Mots-clés: racine, décomposition, cinétique de Michaelis-Menten, pH optimal, temps d'incubation, phosphomonoesterase, ß-glucosidase, α-glucosidase, cellobiohydrolase, ß-xylanase, N-acetyl-ß-glucosamidase, aryl sulphatase, arbres tropicaux, Panama.

2.1 Abstract

Microbial communities use a diverse set of enzymes to degrade plant litter carbon (C) components and acquire nitrogen (N) and phosphorus (P) during various stages of the decomposition process. In spite of the important role of enzymes in plant litter decomposition, studies including this biochemical perspective are scarce and restricted mainly to a handful of studies using leaf litter from temperate ecosystems. Our principal objective in this second chapter was to develop a standard protocol for measuring the enzymatic activity of decomposers in root decomposition studies and demonstrate its ecological application. We suggest parameter values, i.e. substrate concentration, optimal pH, and incubation time based on trials with decomposed material of five native tropical species and a five-species root mixture. Enzymatic activity values associated with C, N and P were used to evaluate the relative importance of these nutrients in decomposition rates over time at two sites with contrasting soil properties in central Panama, Sardinilla and Agua Salud. The field experiment lasted for 485 days and enzymatic activity was measured after 50, 310, and 485 days of decomposition. The studied enzymes related with degradation of C were cellobiohydrolase (CEL), ß-glucosidase (ßG), and ß-xylanase (XYL). Enzymes related to the acquisition of N and P were *N*-acetyl ß-glucosaminidase (NAG) and phosphomonoesterase (PME). For the assays to determine optimal parameters for measuring enzymatic activity, α-glucosidase (AG) and aryl sulphatase (AS) were included as well. Across enzymes, our results indicated that enzymatic activity in decomposed coarse roots should be measured using a substrate concentration of 100-200 µmol and a buffer with a pH of 4.8 to 5. Incubation times varied across enzymes, from 45 min for PME to > 180 min for AG, CEL, XYL and AS. Consistent with our general hypothesis, we observed a relationship between enzymatic activity and root decomposition across species and study sites. Cumulative enzymatic activity predicted between 21 to 63% of decomposition rates. Activity of enzymes related to N and P acquisition, in particular, were strongly correlated with decomposition rates, i.e. faster decomposition was associated with higher cumulative enzyme activity. Activity of enzymes associated with C were related to species identity, although site effects were strong, thus indicating that external factors, i.e. differences in decomposer communities or soil properties, also may modulate decomposition rates. Unexpectedly, cumulative enzymatic activity of C enzymes was not explained by root traits that described C quality. Variation in enzymatic activity related with N was explained principally by soil enzymatic activity, while variation in enzymatic activity associated with P was uniquely explained by root functional traits. Although different factors clearly influence the role of these enzymes during decomposition, the quantification of enzymatic activity allowed us to assess the relative importance of different functional groups of microbial decomposers during the root decomposition process.

Keywords: coarse roots, decomposition, Michaelis-Menten kinetics, optimal pH, incubation time, phosphomonoesterase, ß-glucosidase, α-glucosidase, cellobiohydrolase, ß- xylanase, N-acetyl-ß-glucosamidase, aryl sulphatase, tropical trees, Panama.

2.3 Introduction

Tropical forests play an important role in the global nutrient cycle (Vitousek and Sanford 1986a, Malhi et al. 1999), fixing ~70 % of terrestrial nitrogen (Townsend et al. 2011) and assimilating 60 % of global carbon (Malhi and Grace 2000). Their importance in the global nutrient cycle is closely related to their vast diversity. Plant diversity could influence ecosystem processes involved in the carbon cycle such as productivity and decomposition (Cardinale et al. 2012, Hooper et al. 2012). However, uncertainties about the relative contributions of belowground components to the nutrient cycle still persist (Vitousek and Sanford 1986a, Pan et al. 2011). Better estimations of the belowground contribution in the tropics, that considers their susceptibility to land use and climatic change (McGroddy and Silver 2011, Ngo et al. 2013), are needed to improve models for predicting changes in nutrient cycles at a global scale.

Plant tissues (leaves, roots and wood) are composed of a diversity of carbon (C) components with different levels of C lability that ultimately influence their decomposition rates (Berg and McClaugherty 2008). The fastest degradable and most easily metabolizable C components are monosaccharides, followed by more complex polysaccharides (cellulose and hemicelluloses), and then bio-polymers such as lignin (Baldrian and Snajdr 2011). Due to variation in C quality and content across plant tissue and species in the tropics (Hättenschwiler and Jørgensen 2010, Hättenschwiler et al. 2011), decomposition of plant tissues is a complex process. Therefore, microbial communities involved in organic matter decomposition use specific extra-cellular enzymes to degrade these C components (Baldrian and Snajdr 2011).

Measurements of enzymatic activity can elucidate the role of microbial communities during the decomposition process at varying temporal scales (Sinsabaugh et al. 2002, Caldwell 2005). During the initial stages of leaf litter decomposition, Sinsabaugh et al. (2002) and Baldrian and Snajdr (2011) showed that the greatest concentrations of invertase and α-glucosidase are produced by microbial communities that decompose soluble components. Later, cellulose, ß- glucosidase, and ß-xylosidase

are produced to decompose available polysaccharides. Finally, phenol oxidase and peroxides are generated to decompose lignin. Changes in the duration of each stage of decomposition are associated with the quality of the decomposing litter, as early peaks in cellulose activity are associated with more lignified litter (Sinsabaugh et al. 2002). Despite the usefulness of studying enzymatic activity to understand decomposition of plant material (Sinsabaugh et al. 1991, Sinsabaugh et al. 1994, Moorhead and Sinsabaugh 2000), most studies have been performed on leaf litter in temperate ecosystems while other litter types such as dead wood and roots have been less frequently studied (Sinsabaugh et al. 1993), particularly in tropical ecosystems (Allison and Vitousek 2004).

Extra-cellular enzyme activity of microbial communities is critical to the breakdown of dead organic matter, e.g. lignocelluloses, as well as the release of nutrients such as nitrogen (N), phosphorus (P), and sulfur (S) (Sinsabaugh et al. 2002). Enzymes related to N and P acquisition are associated with nutrient demand and exogenous availability during decomposition (Sinsabaugh et al. 1993). When N and P are not available as inorganic or simple organic compounds, extracellular enzymes are produced. For example, chitin, a complex compound and major reservoir of organic N induces the ß-*N*-acetyl-ß-glucosamidase production to release N (Sinsabaugh et al. 1993, Sinsabaugh et al. 2008). Conversely, when labile N is available, production of ß-*N*-acetyl-ß-glucosamidase is inhibited (Sinsabaugh et al. 1993). P limitation in soils is associated to high productivity of phosphomonoesterase (Sinsabaugh et al. 1993).

Multiple levels of diversity act on decomposition, such as microbial decomposer diversity (Gessner et al. 2010). Microbial groups, e.g. fungus and bacteria, have diverse functional roles during decomposition using enzymatic activity (Boer et al. 2005). Rhee et al. (1987) found that fungi contribute more to the decomposition of cellulose components than bacteria. Gram-positive and gram-negative bacteria are also linked to cellulose activity (Waldrop et al. 2000). On the other hand, ß- glucosidase and ß-xylosidase are linked mainly to gram-negative bacteria (Waldrop et al. 2000). These differences are due to the contrasting abilities of gram-positive bacteria to metabolize

complex substrates, while gram-negative bacteria are stronger competitors for simple substrates (Waldrop et al. 2000). Therefore, evaluating the activity of multiple enzymes could provide further insight to the influence of functional diversity of microbial communities on the decomposition of dead organic matter.

Enzyme production during decomposition is influenced by internal factors, such as litter quality, as well as external factors, such as environmental conditions. During decomposition, litter specific patterns of enzymatic activity are determined by substrate or litter quality, e.g. lignified versus labile litter, tannin content, and particulate size of the material in decomposition (Sinsabaugh et al. 2002, Joanisse et al. 2007). For example, high values of the lignocellulose index in leaf litter (LCI, lignin/lignin + cellulose) have been found to be related to low cellulose activity and slow decomposition rates (Carreiro et al. 2000, Sinsabaugh et al. 2002). Due to differences in litter quality across species, variation in enzymatic activity during decomposition in tropical tree species has been observed (Allison and Vitousek 2004). Soil nutrient availability can also influence the production of enzymes related to decomposition (Sinsabaugh et al. 2002). Studies about the effects of nitrogen deposition on enzymatic activity have shown that high levels of nitrogen can suppress the production of phenol oxidase activity, an enzyme involved in breaking down lignin, thereby reducing decomposition rates of lignified litters in the later stages of decomposition (Carreiro et al. 2000, Hobbie et al. 2012).

Although measurements of a range of enzymes can be used as indicators of microbiological functional diversity, their use and interpretation must be applied with caution (German et al. 2011, Nannipieri et al. 2012). An array of enzyme assay methods needs to be performed to calibrate measurements of enzymatic activity prior to an experiment (Tabatabai and Dick 2002, German et al. 2011). This fundamental step is necessary because enzyme production efficiency varies with biotic and abiotic factors, including pH, substrate selection and substrate quality, resistance of enzymes to environmental conditions, and sorption processes (Sinsabaugh et al. 2002, Taylor et al. 2002, Turner 2010). The enzyme parameters that should be tested include kinetic

variables, affinity of the enzymes for a given substrate, optimal pH, and incubation time (Tabatabai and Dick 2002, German et al. 2011). Kinetic variables and substrate affinities are estimated precisely using the Michaelis-Menten model (Turner et al. 2001, Tabatabai and Dick 2002, German et al. 2011). As well, determining optimal pH values can be used to select an appropriate buffer (German et al. 2011). A range of ecological questions, including leaf decomposition, have been examined using enzymatic activity measurements (Hobbie et al. 2012) However, parameters for measuring enzymatic activity frequently are not measured or reported, increasing methodological concerns about the use of enzymatic activity in different studies (German et al. 2011).

In the present study, we examine activity of multiple enzymes to elucidate the mechanisms that determine coarse root decomposition over time in tropical forests. To this end, our first objective was to define methodological parameters for measuring enzymatic activity of decomposed roots, as these parameters could change across ecosystems and plant substrates. Secondly, we examined activity of multiple enzymes involved in major nutrient cycles (C, N, and P), and later related it to decomposition over time (50, 310 and 485 days of decomposition) providing a framework to understand the dynamics under coarse root decomposition and its ecological implications. We used coarse root material of five tropical tree species established in two sites that differed in soil properties. Based on the diverse functional roles of the studied enzymes, we hypothesized that initial stages of decomposition would be positively correlated with cellobiohydrolase, ß- glucosidase, and ß-xylanase, as found in leaf litter as they have been shown to break down less recalcitrant carbon components (Waldrop et al. 2000, Sinsabaugh et al. 2002, Boer et al. 2005). Activity of enzymes not directly related to decomposition of plant fiber, such as acetyl *N*-acetyl ß-glucosaminidase and phosphomonoesterase, were expected to be correlated with decomposition, given their role in the acquisition of organic nitrogen and phosphorus (Sinsabaugh et al. 2002). Lastly, we explored the extent to which root traits and site conditions influenced enzymatic activity during root decomposition.

2.4 Methods

2.4.1 Study sites and species selection

The study was conducted in two experimental forest sites, Sardinilla (9°19' N - 79°38' W) and Agua Salud (9°13' N - 79°47' W), located in central Panama with annual precipitation of 2350 mm and 2650 mm, respectively (Scherer-Lorenzen et al. 2007, Breugel et al. 2011). Sardinilla is an experimental biodiversity tree plantation established in 2001 where former pasture land was planted in plots of 45 x 45 m with either one, three, or six native tree species (Scherer-Lorenzen. et al. 2005, Scherer-Lorenzen et al. 2007). After 10 years of growth, the Sardinilla plantation has a closed, stratified canopy (Kunert et al. 2011). Understory vegetation is varied and is dominated by grasses and herbaceous species (Potvin et al. 2011). Agua Salud includes secondary forests of >50 years old (Breugel et al. 2011, Paul et al. 2011). While in Sardinilla the soils originated from sedimentary rocks and tertiary limestone, presenting Alfisols dominated by clay (Potvin et al. 2004), in Agua Salud soils originated from pretertiary basalt plateau (Hassler et al. 2011) (see Table 1.2 for soil characteristics).

Roots used to establish the decomposition experiment were harvested from the five species present in Sardinilla. The studied species represent a gradient of relative growth rates, including slow growing species, *Cedrela odorata* and *Tabebuia rosea,* moderate growing species, *Anacardium excelsum* and *Hura crepitans,* and fast growing species, *Luehea seemanii* (Scherer-Lorenzen et al. 2007).

2.4.2 Root material and experimental design

Live coarse roots (2 – 5 mm diameter) corresponding to 4th and 5th order roots from *Anacardium excelsum, Cedrela odorata, Hura crepitans, Luehea seemanii* and *Tabebuia rosea* were excavated, washed, and air dried at 40 °C for four days. Root traits including structural, e.g. specific root length (SRL), root dry matter content (RDMC) and lignin, cellulose, and hemicellulose content, chemical, e.g. carbon (C), nitrogen (N), phosphorus (P) and micronutrients, and anatomical, e.g. cortex, xylem, vessel, and epidermis percent

area, characteristics were measured (see: Chapter 1 for details). Root material was cut to 10 cm in length and 5 grams of each of the five species was placed into 10 x 20 cm nylon decomposition bags with a 2 mm mesh that allowed entry of meso-fauna. As well, a sixth treatment consisting of a five species root mixture where all species were included in equal proportions was included. Root decomposition bags were established in three high diversity plots in Sardinilla (see: Chapter 1 for details) and in three high diversity plots in Agua Salud. High diversity plots in Sardinilla contained all five studied tree species, while those in Agua Salud had approx. 155 species in 0.2 ha (Breugel pers. communication). This design yielded three replicates for each of the six treatments at two types (plantation and secondary forest) of high diversity sites.

Root decomposition bags were established diagonally in the top 20 cm of the soil (see Fig. 1.1, Chapter 1) in August/September 2011. Subsamples for measuring enzymatic activity were collected after 50, 310 and 485 days. To prepare the sub-samples, roots were washed carefully with reverse-osmosis water over a 2 mm and 250 µm mesh sieve. Later, 1 – 2 g of root were taken from each replicate and frozen at -35 °C until analyzed. For five-species root mixtures, the subsamples were taken including small pieces from every remaining roots. Mass loss at each collection date was determined by oven-drying the remaining samples at 65 °C for four days. After 50 and 310 days, fresh mass of the subsamples removed for measuring enzymatic activity was converted to dry mass based on the specific conversion factor between the fresh mass and dry weight of the remaining samples. Later, this dry mass was added to the remaining sample to estimate mass loss. After 485 days, independent samples for mass loss and enzymatic activity were used because there was not sufficient root material for certain species.

2.4.3 Determination of parameters to measure enzyme activity during coarse root decomposition

In order to develop a standardized protocol for measuring enzymatic activity in dead, coarse roots of tropical tree species, enzymatic parameters (kinetics, substrate affinity, optimal pH, and incubation time) were tested for coarse roots after 50 days of

decomposition in Sardinilla. Each parameter was calibrated using three samples of root material, which were selected from the studied species (*Anacardium excelsum*, *Cedrela odorata*, *Hura crepitans*, *Luehea seemanii*, *Tabebuia rosea* and five species root mixture) in order to capture as much variation as possible in substrate characteristics.

2.4.3.1 Enzymatic activity assay

Enzymatic activity of seven enzymes involved in the release of carbon (C), nitrogen (N), phosphorus (P) and sulphur (S) were measured (Table 2.1). Characterization of these seven hydrolytic enzymes was performed with a modified fluorogenic substrate method (Turner and Romero 2010). Enzymatic activity was assessed using 4-methylumbelliferyl (MU) that links compounds to substrate analogues. For each sample, 1 g of fresh decomposed root material was homogenized in a coffee grinder for 30 seconds. Suspensions were then prepared with the ground root material in deionized water (1:100 ratio), plus 1 mM NaN_3 to prevent microbial activity. The suspension was stirred using a magnetic stir-plate for 10 min. Micro-well plates were used to quantify enzymatic activities, including 50 µL of root material, 100 µL of substrate, and 50 µL of sodium acetate acetic acid buffer. Controls for each substrate (containing substrate, buffer and 1 mM NaN_3) and blanks (suspension and buffer) were included. Plates were incubated at 30°C. To stop the reaction, 50 µL of 0.5 M NaOH was added. Fluorescence was measured at an excitation wavelength of 355 nm and an emission wavelength of 450 nm using a computerized fluorimetric microplate reader (Fluostart Optima, BMG Labtech, Offenberg, Germany).

Table 2.1 Substrates used in assays of enzymatic activity, the enzyme required for their breakdown and the main element released.

Element	Enzyme	Substrate	Code
Carbon	α-Glucosidase	4-Methylumbelliferyl α-D-glucopyranoside	AG
	ß-Glucosidase	4-Methylumbelliferyl ß-D-glucopyranoside	ßG
	Cellobiohydrolase	4-Methylumbelliferyl ß-D-cellobiopyranoside	CEL
	ß-Xylanase	4-Methylumbelliferyl ß-D-xylopyranoside	XYL
Nitrogen	N-acetyl-ß-D-glucosaminidase	4-Methylumbelliferyl *N*-acetyl-ß-D-glucosaminide	NAG
Phosphorus	Phosphomonoesterase	4-Methylumbelliferyl phosphate	PME
Sulphur	Aryl sulphatase	4-Methylumbelliferyl sulphate potassium salt	AS

2.4.3.2 Kinetic parameter measurements and calculations

Eight substrates between 2 to 1000 μM were used to calculate kinetic parameters for seven enzymes with root litter. Kinetic assays were made using a buffer at pH 5. PME, ßG, NAG were incubated for 1 h while AG, CEL, XYL, and AS were incubated for 2 h. Kinetic parameters were estimated using a Michaelis – Menten equation:

$$V = \frac{V_{max}\, S}{K_m + S}$$

where the velocity of reaction at any time is V and the substrate concentration by S (Cornish-Bowden 1995). The Michaelis constant (K_m) is an indicator of an enzyme's affinity for a particular substrate (highest affinity is equal to low K_m) wherein the velocity of the reaction increases with the substrate concentration until maximal velocity (V_{max}) (Cornish-Bowden 1995).

Additionally, three contrasting linear transformations of Michelis – Menten equations were used to calculated V_{max} and K_m: Lineweaver-Burn or double-reciprocal, Hanes or Woolf, and Eadie-Hofstee. The Lineaweaver-Burn transformation is based on 1/V against 1/S, where the slope is K_m/V and the intercept is 1/V. Even though this has been used commonly, it can be misleading regarding experimental errors for different V values (Cornish-Bowden 1995). The Hanes transformation is based on S/V against S, where slope is 1/V and the intercept is K_m/V. This transformation provides better experimental error calculations in comparison with the other two transformations (Cornish-Bowden 1995). Lastly, the Eadie-Hofstte transformation is based on V against V/S, where slope is $-K_m$ and the intercept is V. Although the Eadie-Hofstee transformation provides good results, experimental error affects both axes (Cornish-Bowden 1995).

2.4.3.3 Optimal pH values and incubation time

Multiple tests were performed to characterize optimal pH buffers and incubation times using substrates with a final concentration of 100 μM. pH tests were performed using seven sodium acetate acetic acid buffers, ranging from 3.7 to 5.6. Additionally, enzymatic activities were quantified using diverse incubation times, 15, 30, 60, and 180 minutes. A buffer of pH 5 was used for the incubation time assays.

2.4.4 Enzymatic activity and root decomposition

Subsamples from Sardinilla and Agua Salud at 50, 310 and 485 days were analyzed using the enzymatic activity assay explained above (section: 2.3.2.1). Only five of the seven enzymes (ßG, CEL, XYL, NAG, and PME) were analyzed using the instantaneous and cumulative enzymatic values, since values of AG and AS activities were extremely low in the preliminary assay. Instantaneous enzymatic activity was measured after 50, 310, and 485 days in μmol MU g min^{-1}, while cumulative enzymatic activity was calculated as the value under a curve relating enzymatic activity vs. time (Sinsabaugh et al. 2002). Decomposition rates (*k*) were calculated following a first order,

exponential decay model proposed by Olson (1963) using mass loss for four harvest during 485 sampling days. Additionally, enzymatic activity of the same five enzymes was measured on soil collected in the first 20 cm deep inside experimental plots after 485 days. Mass loss was calculated for each sampling period.

2.4.4.1 Data analysis

Relationships between instantaneous and cumulative enzymatic activity and decomposition were evaluated using standard major axis (SMA) regression in the 'smatr' package, as both contain measurement error (Warton et al. 2012). To determine if enzymatic activity varied by species identity of root litter, installation sites, and/or harvest time, repeated measures analysis of covariance (ANCOVA) were performed. All variables had homogeneous variance; logarithmic transformation was used to meet normality assumptions for mass loss values. In addition, principal component analysis (PCA) was used to assess relationships among activity of multiple cumulative enzymes and decomposition rates using the 'vegan' package (Oksanen et al. 2013). Finally, partial least square regression (PLSR) models were fit to examine the extent to which root decomposition rates were explained by enzymatic activity. As well, PLSR models were fit to determine if enzymatic activity was explained by root functional traits, and soil characteristics, i.e. soil enzymatic activity. Model performance was evaluated using cross-validation. Mean squared errors of prediction (MSEP) and root mean square errors (RMSE) were used to select the best fit model. PLSR models were fit using the 'pls' package (Mevik et al. 2011). All statistical analyses were performed using R 2.15.3 (R Development Core Team. 2011).

2.5 Results

2.5.1 Determination of parameters to measure enzyme activity during coarse root decomposition

2.5.1.1 Kinetic parameters

Variations in kinetic parameters such as V_{max} and K_m across species and enzymes were observed (Table 2.2). Across species, the lowest values for V_{max} were 1 and 2 μmol g^{-1} min^{-1} for AS and AG, respectively, while for K_m the lowest values was 4 μmol for CEL. The highest values for V_{max} and K_m were observed for PME with values of 263 μmol g^{-1} min^{-1} and 110 μmol, respectively. Across enzymes, the lowest values for V_{max} and K_m were found in roots of *Tabebuia rosea* and *Hura crepitans,* while the highest values were measured in roots of *Hura crepitans* and the five species root mixture, respectively. Species with lowest and highest values for V_{max} and K_m differed only for PME and AS.

Enzymatic activity of ßG, CEL, and NAG followed Michael-Menten kinetics, while enzymatic activity of AG, XYL, PME, and AS did not (Fig. 2.1, Table 2.2). The Hanes-Woolf transformation revealed that at low and high substrate concentration ranges, two distinct rates of enzyme kinetics were presented (Table 2.3), such as *Hura crepitans* and *Tabebuia rosea* for PME and XYL, respectively (Fig. 2.2). The low concentrations found for the five species root mixture in ßG, AG, and XYL and for *Tabebuia rosea* in AG and XYL ranged between 2 to 200 μmol, while the high concentration range was between 200 – 1000 μmol. Low and high concentration ranges for PME and AS were between 2 – 100 μmol and 100 – 1000 μmol, respectively, except for the five species root mixture in AS where concentrations were between 2 – 500 and 500 – 1000 μmol, respectively. Lower K_m values were found in lower concentration ranges. For example, *Hura crepitans* had a K_m value of 42 μmol in low concentration, but a K_m value of 559 μm in high concentration. Hence, greater affinities for the substrate in low concentrations were associated with low K_m values.

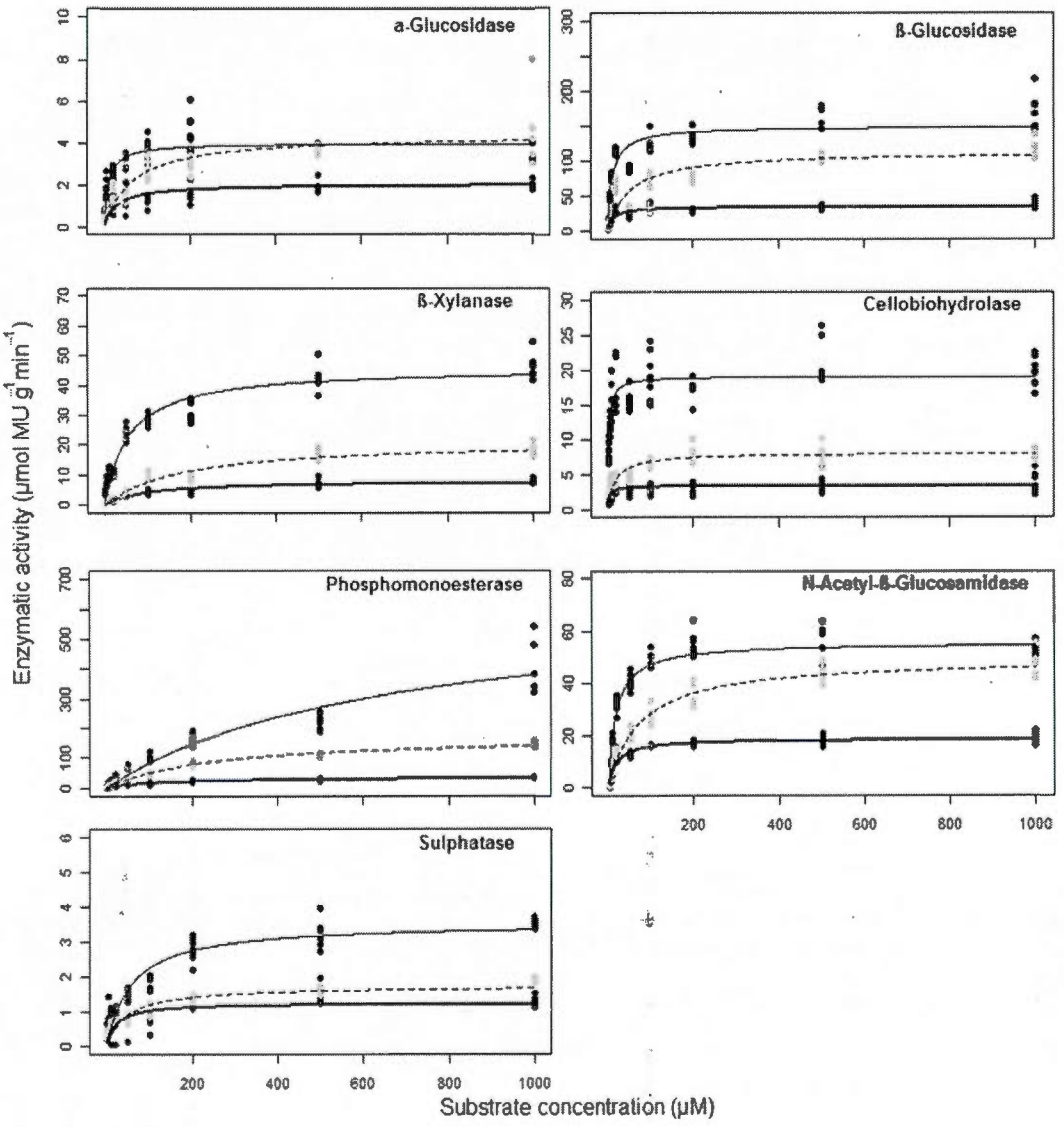

Figure 2.1 Michaelis–Menten kinetics plots. *Hura crepitans* (solid line), *Tabebuia rosea* (thicker line) and the five species root mixture (dotted line).

Table 2.2 Kinetic parameters for roots after 50 days of decomposition in Sardinilla. Two extreme examples (fast decomposing *Hura crepitans* and slow decomposing *Tabebuia rosea*) and one intermediate example (five species root mixture) are shown.

Enzymes	Michaelis-Menten			Lineweaver-Burk			Eadie- Hofstee			Hanes-Woolf			Mean*	
	V_{max}†	K_m‡	Residual	V_{max}†	K_m‡	R^2	V_{max}†	K_m‡	R^2	V_{max}†	K_m‡	R^2	V_{max}†	K_m‡
ß-glucosidase														
Hura crepitans	149.8	11.5	19.9	173.2	16.9	0.97	146.3	10.9	0.71	167.1	25.4	0.98	162.2	17.7
Tabebuia rosea	34.5	13.4	5.4	44.2	26.1	0.96	33.0	12.8	0.56	38.1	32.0	0.97	38.4	23.6
Root mixture	113.5	50.8	16.5	93.5	28.9	0.88	89.2	18.6	0.38	124.6	74.3	0.97	102.4	40.6
α-glucosidase														
Hura crepitans	4.0	10.0	0.6	4.2	12.3	0.91	3.7	7.3	0.57	4.0	11.6	0.99	4.0	10.4
Tabebuia rosea	2.0	29.4	0.4	1.5	8.4	0.82	1.6	7.2	0.41	2.1	43.4	0.94	1.7	19.7
Root mixture	4.4	66.4	0.7	2.7	15.4	0.87	3.2	16.2	0.49	4.3	57.3	0.92	3.4	29.6
Cellobiohydrolase														
Hura crepitans	19.1	2.7	2.6	18.4	2.5	0.81	18.7	2.3	0.55	19.7	5.8	0.98	18.9	3.5
Tabebuia rosea	3.3	4.4	0.8	3.0	3.7	0.68	3.2	3.2	0.30	3.4	15.2	0.88	3.2	7.4
Root mixture	8.0	18.2	1.1	5.8	5.0	0.79	6.7	6.2	0.54	8.2	20.3	0.98	6.9	10.5
ß-xylanase														
Hura crepitans	45.9	55.5	4.0	26.8	12.1	0.92	35.4	18.7	0.67	47.8	56.3	0.98	36.7	29.0
Tabebuia rosea	8.1	102.6	0.8	3.7	26.9	0.70	5.1	29.6	0.44	8.3	104.4	0.94	5.7	53.6
Root mixture	21.4	179.1	1.5	4.4	20.4	0.76	13.0	63.8	0.44	20.8	172.9	0.94	12.7	85.7
***N*-acetyl-ß-glucosaminidase**														
Hura crepitans	55.5	17.5	4.4	61.4	25.1	0.98	56.4	19.9	0.86	52.2	11.5	0.99	56.7	18.8
Tabebuia rosea	18.6	19.2	1.6	22.7	34.2	0.92	18.1	18.6	0.68	18.4	20.8	0.98	19.7	24.5
Root mixture	49.5	72.8	3.0	47.8	59.8	0.97	44.6	51.2	0.84	49.8	71.1	0.99	47.4	60.7
Phosphomonoesterase														
Hura crepitans	632.0	661.4	35.6	137.4	37.4	0.99	245.8	78.8	0.57	404.4	214.1	0.82	262.5	110.1
Tabebuia rosea	36.4	152.7	2.5	12.7	18.2	0.65	21.8	35.4	0.48	35.7	123.9	0.96	23.4	59.2
Root mixture	174.7	247.6	6.3	104.8	74.5	0.98	115.0	82.1	0.75	161.0	164.7	0.97	126.9	107.1
Sulphatase														
Hura crepitans	3.5	57.2	0.4	2.1	8.4	0.82	2.4	9.8	0.49	3.7	49.5	0.98	2.7	22.6
Tabebuia rosea	1.2	23.1	0.3	0.7	3.8	0.21	1.0	4.9	0.19	1.4	89.2	0.80	1.0	32.6
Root mixture	1.7	46.1	0.2	1.0	4.7	0.67	1.2	5.1	0.34	1.9	52.2	0.99	1.4	20.7

Notes: *Mean of Lineweaver-Burk, Eadie-Hofstee, and Hanes-Woolf transformations, †V_{max}: μmol g^{-1} min^{-1}; ‡K_m: μmol.

Table 2.3 Kinetic parameters for roots calculated using the Hanes Woolf transformation. Two-phase kinetics was found for phosphomonoesterase and ß-xylanase at low and high substrate concentrations

Enzymes	Species	Substrate range µmol	K_m µmol	V_{max} µmol g^{-1} min^{-1}
ß-glucosidase (ßG)	Root mixture	2 – 200	33	161
		200 – 1000	89	138
α-glucosidase (AG)	*Tabebuia rosea*	2 – 200	15	2
		200 – 1000	94	2
	Root mixture	2 – 200	31	3
		200 – 1000	118	5
ß-xylanase (XYL)	*Tabebuia rosea*	2 – 200	36	4
		200 – 1000	338	11
	Root mixture	2 – 200	106	14
		200 – 1000	265	23
Phosphomonoesterase (PME)	*Hura crepitans*	2 – 100	42	137
		100 – 1000	559	565
	Tabebuia rosea	2 – 100	28	18
		100 – 1000	220	40
	Root mixture	2 – 100	46	68
		100 – 1000	310	187
Aryl sulphatase (AS)	*Hura crepitans*	2 – 100	12	2
		100 – 1000	94	4
	Tabebuia rosea	2 – 100	51	1
		100 – 500	149	2
	Root mixture	2 – 500	34	2
		500 – 1000	225	2

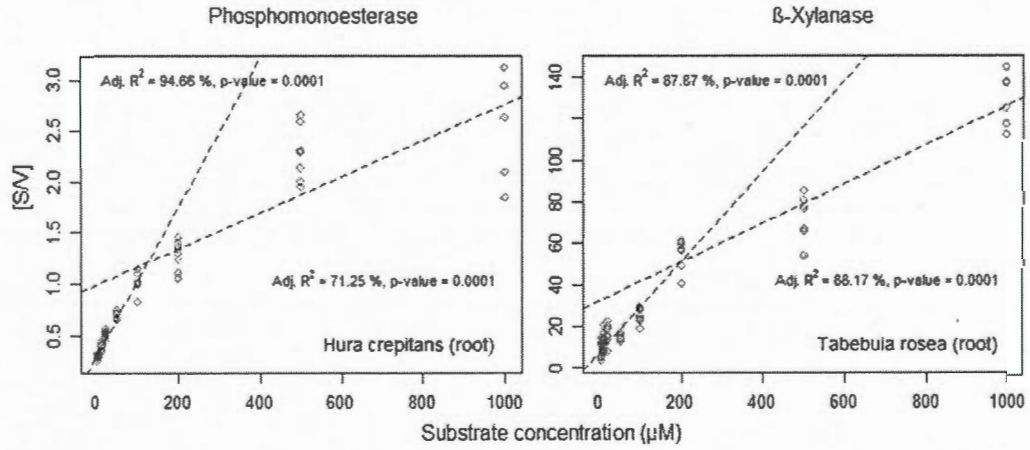

Figure 2.2 Hanes-Woolf kinetics plot showing rates of reaction under low and high substrate concentrations of phosphomonoesterase (2 – 100 and 100 – 1000 µm) for *Hura crepitans* and ß- xylanase for *Tabebuia rosea* (2 – 200 and 200 – 1000 µm).

2.5.1.2 Optimal pH values and incubation time

Considerable intra-specific variation in enzymatic activity was observed when using contrasting pH values. Nonetheless, the optimal pH value was consistently between 4.8 and 5.0 across species and enzymes (Fig. 2.3). *Hura crepitans* was the species with the highest enzymatic activity as well as the highest variation of enzymatic activity across pH values. For example, CEL in *Hura crepitans* varied between 2 to 12 µmol MU g^{-1} min^{-1} at 3.7 and 5.0 pH values, while CEL in *Tabebuia rosea* varied between 0.9 to 1.8 µmol MU g^{-1} min^{-1} at 4.8 and 5 pH values, respectively. *Anacardium excelsum* had a similar pattern as *Tabebuia rosea* for CEL. Across species, two optimal pH values were observed for AG and AS, 3.7 and 4.8/5.0.

The maximum values for enzymatic activity after the longest incubation time (180 minutes) by enzyme, from lowest to highest, was PME > BG> NAG> CEL> XYL> AG>AS. Although across samples, enzymatic activity did not exceed 30,000 µmol MU g^{-1} after 180 minutes of incubation (Fig. 2.4), two distinct patterns were observed. First, the values of AS and AG were lowest and difficult to detect. Secondly,

the highest PME values (>65,000 μmol MU g^{-1} after one hour) could be found when other species and/or incubation sites are used (data not show).

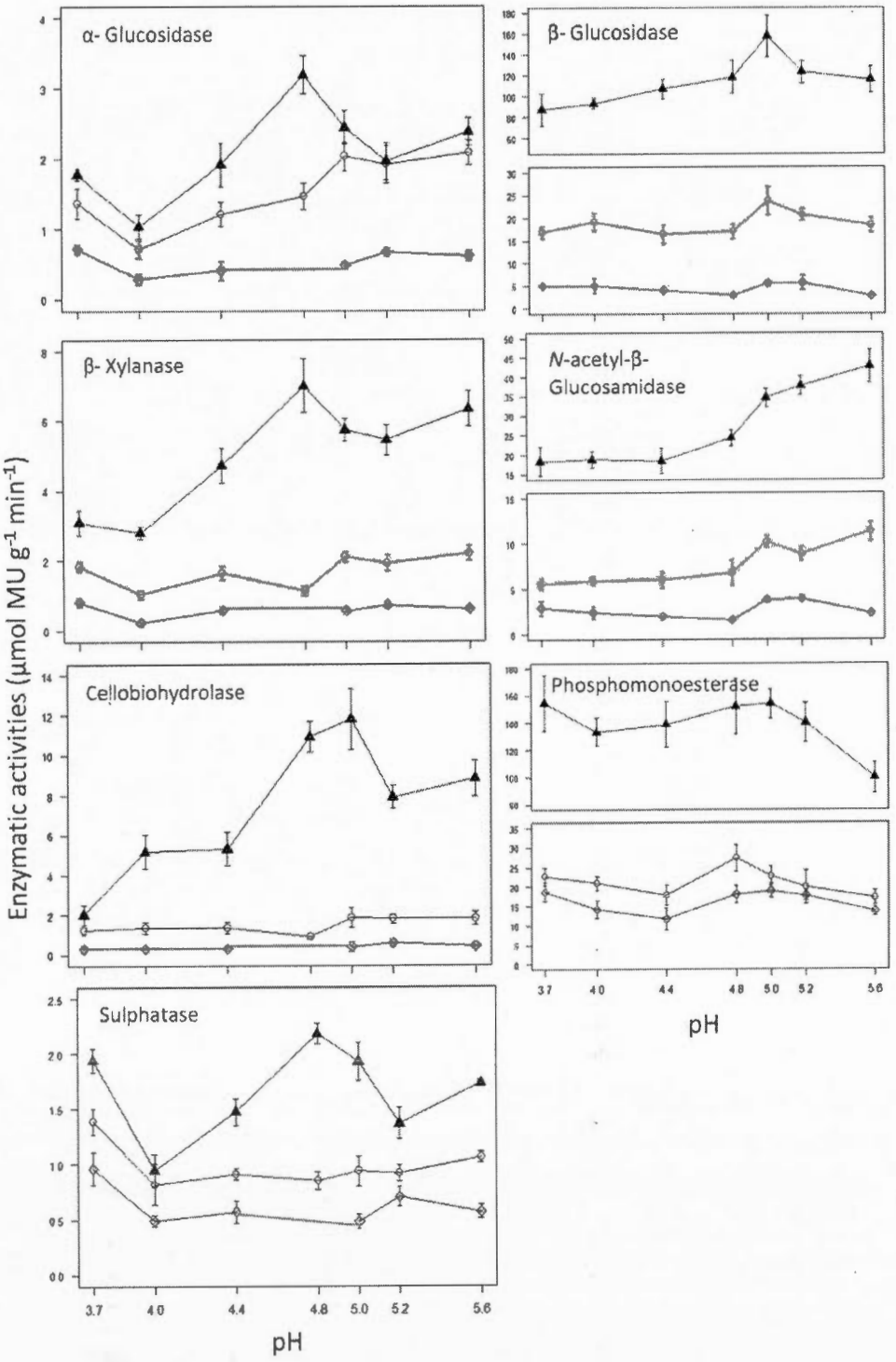
α- Glucosidase
β- Glucosidase
β- Xylanase
N-acetyl-β-
Glucosamidase
Cellobiohydrolase
Phosphomonoesterase
Sulphatase
Enzymatic activities (µmol MU g-1 min-1)
pH
pH

Figure 2.3 Enzymatic activity of roots after 50 days of decomposition under varying buffer pH conditions. Empty circles (*Tabebuia rosea*), gray diamond (*Anacaridum excelsum*), and black triangles (*Hura crepitans*).

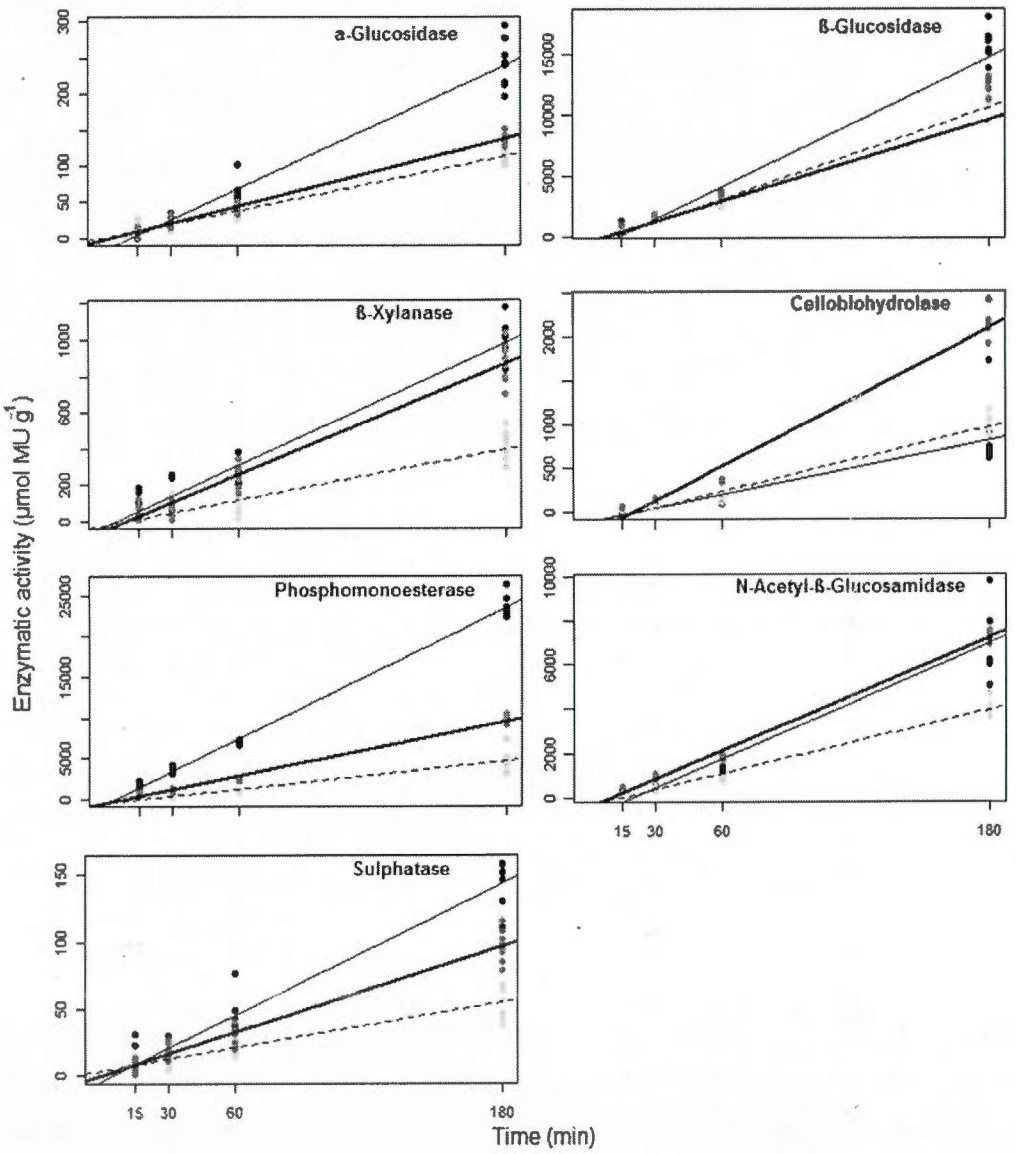

Figure 2.4 Enzymatic activity measures after 15, 30, 60 and 180 minutes of incubation. *Hura crepitans* (solid line), *Anacardium excelsum* (dotted line), and *Luehea seemanii* (thicker line)

2.5.2 *Enzymatic activity and root decomposition*

Significant, but at times weak relationships between decomposition and enzymatic activity were found across sites. Between cumulative enzymatic activity and decomposition rates, coefficients of variation (R^2) varied between 22 to 56 %, while between instantaneous enzymatic activity and mass loss, they ranged between 4 to 27 %. When enzymatic activity from both sites was analyzed together, only XYL was marginally correlated with mass loss (Fig. 2.5), while significantly positive correlations were found for enzymes related to N and P acquisition (Fig. 2.6). When sites were considered separately, significantly positive relationships between enzymes for C, N, and P acquisition and decomposition rates were observed in Sardinilla ($p<0.05$), but not for cumulative values of CEL (Fig. 2.5). In contrast, significant negative relationships for ßG and CEL with mass loss were observed in Agua Salud ($p<0.05$). Furthermore, mass loss and cumulative values of NAG and PME exhibited significant and positive correlations in Agua Salud; instantaneous NAG activity, however, was not correlated significantly with mass loss (Fig. 2.6).

Across study sites, activity of multiple enzymes strongly regulated decomposition rates of roots. PLSR models using enzymatic activity as explanatory variables explained 63 % of decomposition in Sardinilla, 21 % in Agua Salud, and 38 % when both sites were analyzed together. In Sardinilla, enzymes that significantly explained decomposition, in order of their standardized coefficient sizes, were: ßG> PME> XYL> CEL. In Agua Salud, decomposition was only explained by PME with a standardized effect size of 0.550, while across sites it was predicted significantly by PME (standardized coefficient size = 0.441) and NAG (standardized coefficient size = 0.375) (Table S2.1 and S2.2).

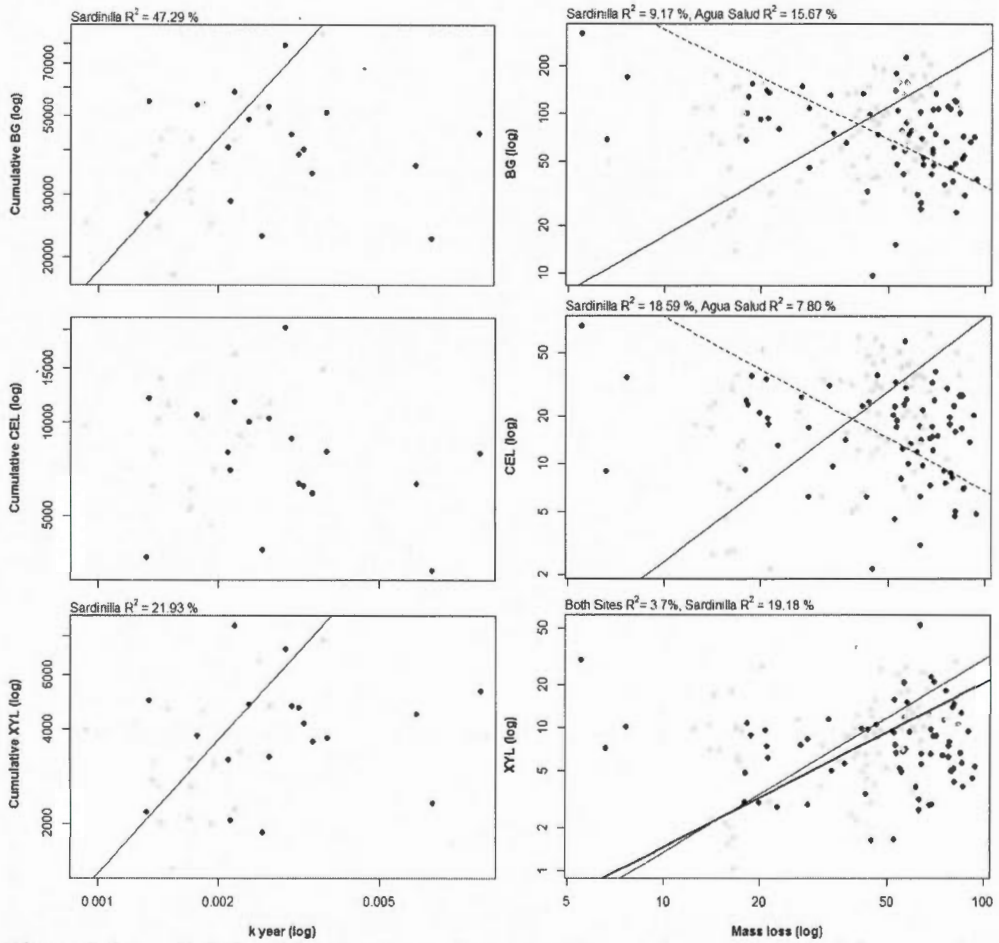

Figure 2.5 Relationships between decomposition and enzymatic activity associated with carbon. The left column shows cumulative enzymatic activity versus the annual *k* constant and the right column shows enzymatic activity (μmol MU g min^{-1}) versus mass loss across all species. Lines represent significant relationships ($p<0.05$) in Sardinilla (solid line), Agua Salud (dotted line), and both sites (thick line). Sardinilla samples are represented by yellow points and Agua Salud by blue points. ßG: ß-glucosidase, CEL: cellobiohydrolase, and XYL: ß-xylanase.

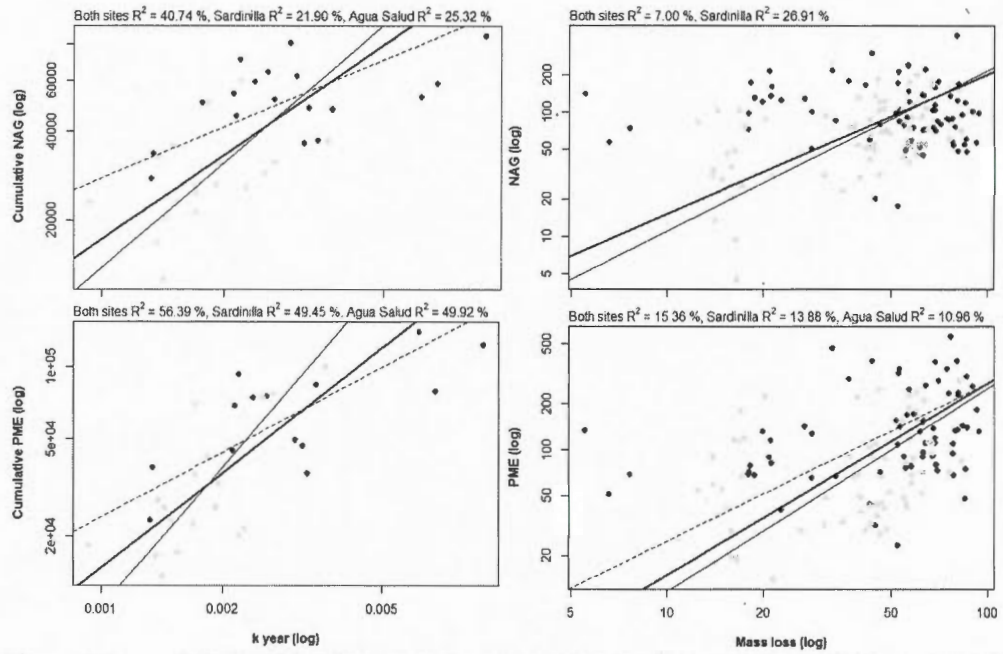

Figure 2.6 Relationships between decomposition and enzymatic activity associated with nitrogen and phosphorus acquisition. The left column shows cumulative enzymatic activities versus the annual *k* constant and the right column shows enzymatic activity (μmol MU g min^{-1}) versus mass loss across all species. Lines represent significant relationships ($p<0.05$) in Sardinilla (solid line), Agua Salud (dotted line) and both study sites (thick line). Sardinilla samples are represented by yellow points and Agua Salud by blue points. NAG: *N*-acetyl-ß-D-glucosaminidase and PME: phosphomonoesterase.

2.5.2.1 Effects of species and site on enzymatic activity during root decomposition

Decomposition rates and cumulative enzymatic activity varied significantly across species and sites (Fig. 2.7). *Hura crepitans* was the species with the fastest decomposition in Sardinilla (k=1.1g $year^{-1}$) and in Agua Salud (k=2.6 g $year^{-1}$). High decomposition rates in Sardinilla were linked to high production of enzymes related to C, while in Agua Salud high decomposition rates were associated with activity of enzymes related to P and N acquisition. In the PCA, samples were strongly grouped by site, with the exception of *Hura crepitans* in Sardinilla, thus substantiating site differences enzymatic activity and

decomposition rates. *Anacardium excelsum* in Agua Salud was the only species that was related more strongly to C enzymes than to NAG and PME enzymes.

The influence of species site and their interactions on enzymatic activity during decomposition was further supported by the results of the repeated measures ANCOVA (Table 2.4). Across enzymes, the interaction between species and site was significant ($p<0.03$), excluding PME, where species was the only significant variable ($p<0.001$). As a main effect, harvest time did not significantly predict variation in enzymatic activity. However, the interaction of species, site, and harvest time was significant ($p\leq0.05$) for CEL, XYL, and NAG, and was marginally significant for ßG ($p = 0.06$). In addition, variation in XYL was explained significantly by the interaction between species and harvest time ($p=0.02$), while that of NAG was explained by site ($p <0.01$) and the interaction between site and harvest ($p =0.03$).

Fitted PLSR models using root traits and soil enzymatic activity explained 57% and 75% of variation in NAG and PME, respectively. The model for NAG was mainly explained by enzymatic activity in soil, while root traits principally explained variation in PME (Fig. 2.9, Table S2.3). High NAG activity was related to low values of ßG, CEL, XYL, and NAG in soil and low lignin, vessel, and sodium (Na) content and high values of PME in soil and high SRL, aluminum (Al), and manganese (Mn) content. High PME activity was associated with low values of ßG, CEL activity in soil and RMDC, lignin, cortex, vessel, C:N, C:P and calcium (Ca) content and with high values of PME in soil and, SRL, hemicelluloses, cellulose, ash, Al and Mn. Models for activity of enzymes related to C acquisition exhibited poor fit, as they had negative R^2 values (Table 2.5) indicating that neither of the variables used in the model explained cumulative C enzymatic activity during root decomposition.

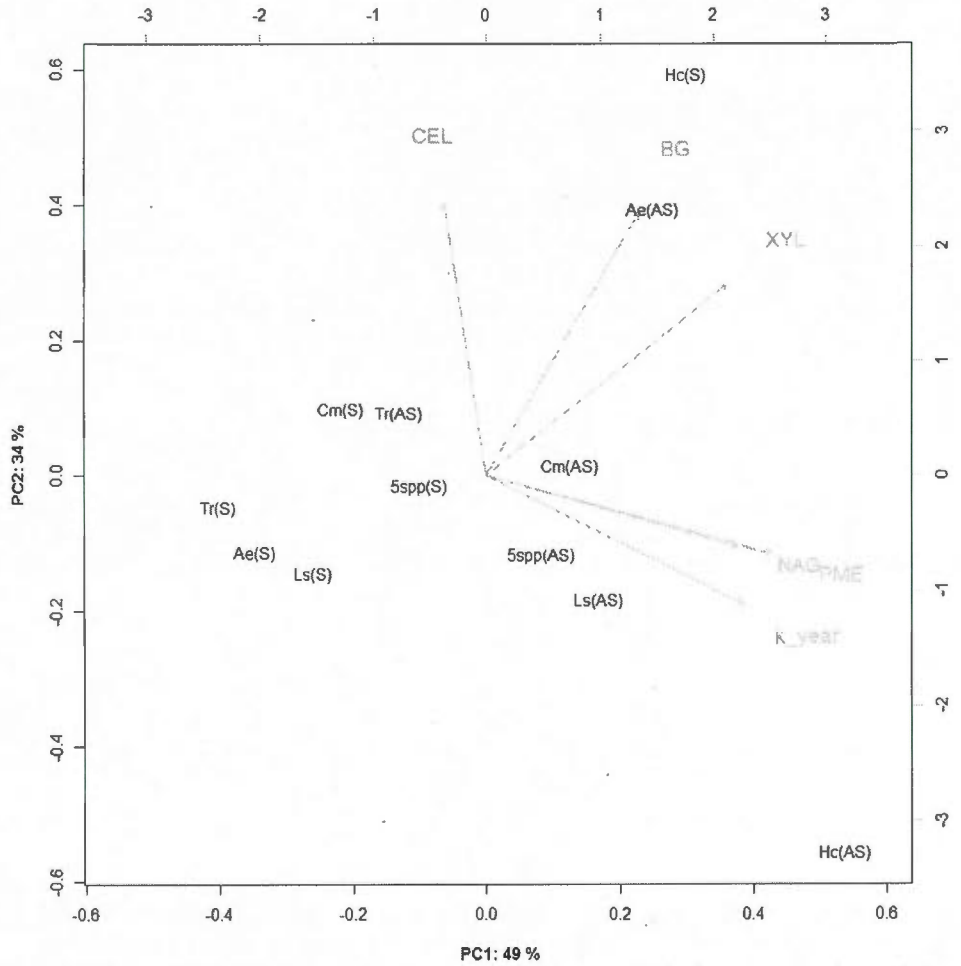

Figure 2.7. Principal component analysis using cumulative enzymatic activity and decomposition rates. The enzymes (in gray) were: ßG: ß-glucosidase, CEL: cellobiohydrolase, XYL: ß-xylanase, NAG: *N*-acetyl-ß-glucosaminidase and PME: phosphomonoesterase. Decomposition is k_year^{-1}. In black, letters refer to the studied species: Ae: *Anacardium excelsum,* Cm: *Cedrela odorata,* Hc: *Hura crepitans,* Ls: *Luehea seemanii* and Tr: *Tabebuia rosea.* Inside the parentheses, letters refer to study site: S: Sardinilla and AS: Agua Salud.

Table 2.4 Results of the repeated – measures ANCOVA using instantaneous values of enzymatic activities.

Variable	Df	ßG		CEL		XYL		NAG		PME	
		F	p	F	P	F	P	F	p	F	p
Species composition	5	0.58	0.716	0.26	0.932	2.47	0.041	1.91	0.103	4.70	**<0.001**
Site	1	0.17	0.678	0.45	0.503	0.02	0.898	8.39	**0.005**	0.69	0.410
Harvest	2	2.80	0.067	0.49	0.616	0.53	0.592	0.67	0.513	0.16	0.851
Species * Site	5	4.38	**0.002**	3.41	**0.008**	5.16	**<0.001**	2.70	**0.027**	1.16	0.336
Species * Harvest	10	0.96	0.484	0.69	0.730	3.16	**0.002**	1.23	0.289	1.77	0.084
Site * Harvest	2	1.22	0.301	0.63	0.534	1.33	0.270	3.55	**0.034**	0.10	0.907
Species * Site * Harvest	10	1.93	0.056	1.95	**0.05**	3.31	**0.001**	2.09	**0.037**	1.40	0.199

Notes: Species composition included five individual root species and a five species root mixture. Data from two sites, Sardinilla and Agua Salud, were used at 50, 310 at 485 days of root decomposition. ßG: ß-glucosidase, CEL: cellobiohydrolase, XYL: ß-xylanase, NAG: *N*-acetyl-ß-glucosaminidase and PME: phosphomonoesterase.

Table 2.5 Partial least squares regression models using root traits, soil enzymatic activity, and their combined effects for explaining cumulative enzymatic activity.

Variable	Model	Component number	MSEP†	RMSE†	R^2
ß-glucosidase (ßG)	Root	1	1.10	1.05	-14.1
	Soils	2	0.97	0.98	-0.2
	Root and Soils	1	1.13	1.06	-16.7
Cellobiohydrolase (CEL)	Root	1	1.23	1.11	-27.6
	Soils	2	0.92	0.96	**4.5**
	Root and Soils	3	1.23	1.11	-27.6
ß-xylanase (XYL)	Root	1	1.10	1.05	-13.6
	Soils	1	1.05	1.03	-9.0
	Root and Soils	1	1.11	1.05	-14.4
N-acetyl-ß-glucosaminidase (NAG)	Root	1	1.03	1.01	-6.0
	Soils	1	0.51	0.72	**46.9**
	Root and Soils	2	0.41	0.64	**57.2**
Phosphomonoesterase (PME)	Root	2	0.50	0.71	**47.9**
	Soils	1	0.86	0.93	**11.0**
	Root and Soils	3	0.24	0.49	**74.9**

Abbreviations: MSEP: Mean squared error of prediction and RMSE: Root mean square error. Bold R^2 values show fitted models, while negative R^2 show unfitted models.

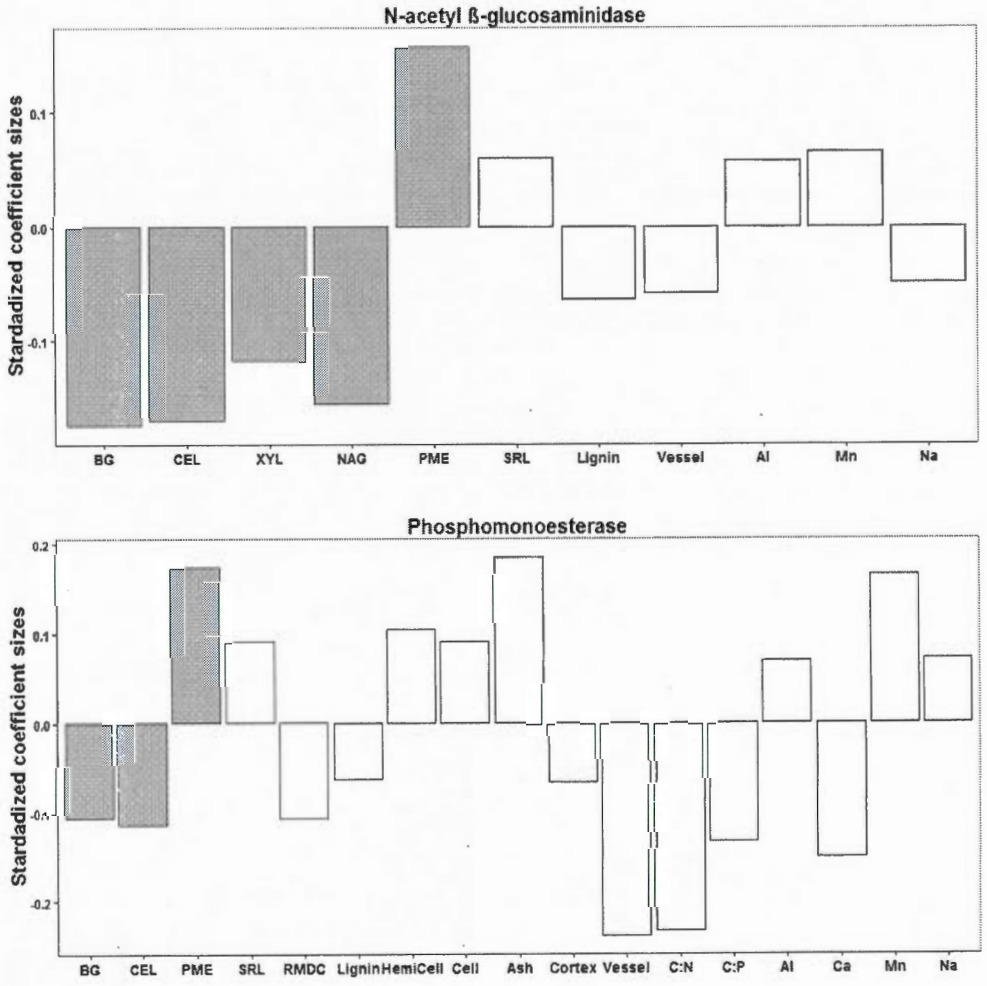

Figure 2.8 Standardized coefficient sizes of partial least squares regression models using root traits (white) and soil enzymatic activy (gray) to explain NAG and PME based on cumulative values. Abbreviations soil enzymes: ßG : ß-glucosidase, CEL: cellobiohydrolase, XYL: ß-xylanase, NAG: *N*-acetyl-ß-glucosaminidase, and PME: phosphomonoesterase. Abbreviations root characteristics: SRL: specific root length, RMDC: root matter dry content, Hemicell: hemicelluloses, Cell: cellulose, Al: aluminum, Ca: calcium, Mn: manganese, and Na: Sodium.

2.6 Discussion

To further our current understanding of temporal variation of coarse root decomposition in tropical tree species, we determined the optimal parameters for measuring activity of enzymes involved in C, N, and P cycles and evaluated how activity of these enzymes varied with respect to decomposition rates over time. Using results from the present study, we provide guidelines for measuring enzymatic activity of coarse roots and then discuss the ecological implications of temporal variation in decomposition and nutrient cycles.

2.6.1 Parameters to measure enzymatic activity in coarse root decomposition

Our results showed that, despite variation of enzymatic activity parameters across and within species, consistent optimal values across species for measuring enzymatic activity could be determined. Consequently, measurements of enzymatic activity will allow for comparisons across decomposed coarse root material of tropical tree species using similar substrate concentration, pH buffer, and incubation times.

The optimal substrate concentration for all enzymes tested ranged between 100 – 200 μmol (Table 2.3). The lone exception to this was AS when testing the five species root mixture, which had an optimal substrate concentration of 500 μmol (Table2.3). Using the five species root mixture permitted us to compare optimal substrate concentrations of decomposed root material of single species versus that of multiple species. The higher substrate affinity found across species and enzymes at low substrate concentrations (100 – 200 μmol) and over a small range of values, could be a result of the normal enzyme quantities, i.e. low concentrations, found in natural environments (Turner et al. 2001).

Our results show that the most frequent optimal pH values across species and enzymes was 4.8/5. The importance of selecting an optimal pH to make comparisons across species was illustrated by the high intra-specific variation of enzymatic activity we observed when using different pH values. An increase in PME activity of 35 % for *Hura*

crepitans, 37 % for *Tabebuia rosea*, and 38 % for *Anacardium excelsum* was observed when optimal pH values were compared with sub-optimal ones (5 vs 5.6, 5 vs 4.4, and 5 vs 5.6, respectively). As well, the increase in CEL activity was 50 % for *Anacardium excelsum* and *Tabebuia rosea* and 83 % for *Hura crepitans* comparing CEL activity when using optimal and sub-optimal pH. Variation in enzymatic activity measurements found in this study when using contrasting pH values is congruent with that found in diverse tropical soils by Turner (2010).

As expected, enzymatic activity increased with incubation for all enzymes across decomposed root material of the studied tree species. Due to the low activity of CEL, XYL, AG, and AS, we used prolonged incubation times of 180 minutes or greater. Using prolonged incubation times for these enzymes will reduce the error associated with blank subtraction when calculating enzymatic activity. In contrast, the high activity of PME permitted shorter incubation times to be used (45 min – 60 min) relative to the other enzymes tested. For this enzyme, short incubation times will enable precise estimates of maximum enzymatic activity to be captured by the computerized fluorimetric microplate reader. By adjusting optimal incubation times for each enzyme, we were able to measure precisely and consistently enzymatic activity of coarse root litter for tropical tree species.

2.6.1.1 Recommendations for measuring enzymatic activity in coarse root decomposition

In the present study, we used coarse roots of tropical tree species that varied widely in terms of structure, anatomy, and chemical content. Thus, it is possible to make recommendations for optimizing the measurement of enzymatic activity as part of future studies on coarse root decomposition in tropical forests:

1) Generally, a substrate concentration between 100 – 200 μmol is appropriate for measuring enzymatic activity in decomposed coarse roots;

2) optimal pH values for the studied enzymes ranged between 4.8 and 5, and;

3) incubation times should be determined separately for each enzyme; they were 45 – 60 min for PME, 60 min for ßG and NAG, and 180 min and more for AG, CEL, XYL and AS.

2.6.2 Enzymatic activity and coarse root decomposition

Consistent with our hypothesis, enzymatic activity played an important role during coarse root decomposition of tropical tree species, predicting 21 and 63% of variation of decomposition in Agua Salud and Sardinil'a, respectively. Unexpectedly, cumulative enzymatic activity of C was not explained by root traits that described C quality. Furthermore, consistent patterns across sites for C enzymatic activity were not found, indicating that external factors, such as differences in decomposer communities or soil properties, also could modulate decomposition. Following expectations, enzymatic activity related to N and P acquisition were strongly correlated with decomposition rates across sites. These results suggest that while enzymatic activity could provide complementary information for understanding root decomposition from a more mechanistic perspective, the complexity of this process due to abiotic conditions and species-specific root traits could hinder the determination of clear patterns.

2.6.2.1 Enzymatic activity associated with C degradation

Our results support the idea that enzymatic activity varies over time during coarse root decomposition. Usually, variation in enzymatic activity related to C degradation has been associated with substrate C quality, e.g. cellulose, hemicelluloses, and lignin content (Sinsabaugh et al. 2002), but our results suggest that abiotic site conditions strongly influenced changes in enzymatic activity over time. Across species, the highest CEL activity in Sardinilla was found after 485 days, with the exception of *Tabebuia rosea* after 310 days, while in Agua Salud, CEL activity peaked after 50 days, except for *Anacardium excelsum* which peaked after 310 days (Table S2.4). Faster decomposition and earlier enzymatic activity peaks in Agua Salud than in Sardinilla contradicted hypotheses like that of 'home-field advantage' where tree overstory

composition could promote more efficient decomposers to break down local litters (Makkonen et al. 2012), as the coarse roots used in the study were sampled in Sardinilla. Across-site variation could be related to differences in soil nutrient properties and microbial decomposer communities, as the most obvious difference between sites was soil type (see Table 1.2), which could result in differences in soil communities due to contrasting soil nutrient content (Wakelin et al. 2012, Jangid et al. 2013). In other words, Sardinilla could have a microbial community formed primarily by bacteria, which are associated with fertile soils, while the microbial decomposer communities in Agua Salud are likely dominated by fungus, which is associated with infertile soils (Wardle et al. 2004). As fungi are more efficient at cellulose degradation (Rhee et al. 1987), early and intermediate stages of decomposition could be faster in Agua Salud than in Sardinilla. Still, this hypothesis needs to be considered with caution as we did not measure directly soil community composition.

2.6.2.2 The role of enzymatic activity related to N and P acquisition on coarse root decomposition

While decomposition of plant nutrients usually has focused on C degradation, results from the present study clearly show that enzymatic activity associated with N and P release also play a fundamental important role in root decomposition in tropical ecosystems. Regarding NAG, greater activity during decomposition was associated with low values of ßG, CEL, XYL, and NAG and high values of PME in the soil. To a lesser extent than enzymatic activity in soil, root functional traits like SRL, lignin, vessel area, Al, Mn, and Na content explained variation in NAG. The relationship between NAG activity and enzymatic activity of multiple enzymes in soil is expected, as enzymes for N are closely related to nutrient demand and exogenous N availability during decomposition (Sinsabaugh et al. 1993). Still, defining the role of N in root decomposition is problematic due to complex N sources and their interactions with other nutrients like P (Sinsabaugh et al. 1993). For example, C:N predict root decomposition rates of tree tropical roots (see: Chapter 1), yet initial root N content was not related to NAG activity. Also, our study showed a significantly positive relationship

between NAG activity and PME with decomposition rates, suggesting complementary interactions between N and P cycles.

Faster decomposition associated with higher PME production found in our study suggested that coarse roots could be an important source of P facilitating C degradation. Cumulative enzymatic activity of PME was explained mainly by root functional traits. High values of PME were associated with high values of SRL, hemicellulose, cellulose, ash, Al, Mn, and Na root content and with low values of RMDC, cortex area, vessel area, C:N and C:P. These same root traits also significantly predicted variation in decomposition rates in our study (see: Chapter 1). The importance of PME, due to highest cumulative values as well as a predictor of decomposition, was stronger in Agua Salud than in Sardinilla. This could be related to the lower available P in Agua Salud than in Sardinilla, as decomposition depends on the ability of plant and microbial communities to use different sources of P (Turner 2008), including root P content. Hence, plant tissues could be a source of other growth-limiting nutrients in the environment, such as P in tropical lowland forests (Vitousek and Sanford 1986b).

Enzymatic activity for diverse nutrients provides insight to the complexity of root decomposition, much of which is likely related to the heterogeneity in environmental conditions. For instance, fast decomposition was related to high cumulative C enzymatic activity in Sardinilla, while in Agua Salud it was associated with high enzymatic activity of NAG and PME. Sinsabaugh and Moorhead (1994) proposed that when inorganic forms of N and P are available, the energy saved by producing enzymes will be relocated and invested in C degradation, i.e. greater production of C enzymes (Sinsabaugh and Moorhead 1994). Although this partly supports the results found in Sardinilla, it does not support the results found in Agua Salud where high values of PME and NAG were associated with faster decomposition.

Plants produce enzymes as a mechanism to obtain nutrients such as P (Vance et al. 2003, Turner 2008), thereby exerting direct effects on processes like decomposition. While evidence for tree overstory diversity effects on decomposition rates and enzymatic

activity was not found (Chapter 1), diversity and composition of understory vegetation still could have affect coarse root decomposition and enzymatic activity. For example, understory vegetation could influence decomposition directly by producing enzymes such as phosphomonoesterase to release phosphate (Turner 2008) and root exudations to obtain diverse N forms (Jones et al. 2005). Contrasting plant understory functional groups, such as grasses, legumes and herbs, could influence decomposers communities, e.g. reproductions and growth rates of invertebrate decomposers (Milcu et al. 2006). Indirect effects of understory vegetation also include the effects of roots on physical and chemical soil components such as soil structure and pH (Ehrenfeld et al. 2005), which are key factors in regulating enzymatic diffusion and efficiency (Sinsabaugh et al. 2002, Taylor et al. 2002, Turner 2010).

2.7 Conclusions

Enzymatic activity plays an important role for understanding the temporal dynamic on root decomposition. To our knowledge, this study is the first to provide specific parameters for measuring enzymatic activity on coarse root decomposition for tropical tree species. We found that optimal values of enzymatic activity parameters varied considerably across enzymes, which suggests that future studies should explicitly determine (and present) these parameters for each enzyme. Establishing a standard protocol for the measurement of enzymatic activity in coarse root litter is a fundamental step in the development of decomposition studies, as it will greatly facilitate comparisons across studies.

Our study established a link in the temporal variation in enzymatic activity of coarse roots during decomposition to variation in abiotic conditions and substrate diversity. The importance of environmental heterogeneity in determining patterns of root decomposition in tropical ecosystems (Chapter 1) was supported by the across-site variation in enzymatic activity that we observed in the present study. In addition to the heterogeneous site conditions, high substrate diversity, i.e. root functional traits, also influenced temporal variation in enzymatic activity. Finally, the complementary

relationship between enzymatic activity related to N and P acquisition and faster decomposition rates found in our study indicates that current biochemical models, developed for temperate species and ecosystems, need to be restructured to account for the complex interactions that arise in diverse tropical ecosystems.

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2.9 Supplementary material

Table S2.1 PLSR models using cumulative enzymatic activity during decomposition to explain root decomposition rates.

Model	Component number	MSEP	RMSEP	R2
Sardinilla	1	0.367	0.606	62.6
Agua Salud	2	0.875	0.936	21.3
Both	1	0.662	0.814	38.4

Abbreviations: MSEP: Mean squared error of prediction and RMSE: Root mean square error.

Table S2.2 Standardized coefficients for PLSR models explaining decomposition rates. Explanatory variables were cumulative enzymatic activities during root decomposition. Only significant coefficients (p-values <0.05) were reported.

Cumulative Enzymatic activity	k constant		
	Sardinilla	Agua Salud	Both
ß-glucosidase	0.292		
Cellobiohydrolase	0.150		
ß-xylanase	0.239		
N-acetyl-ß-glucosamidase			0.375
Phosphomonoesterase	0.285	0.550	0.441

Table S2.3 Standardized coefficients for PLSR models explaining cumulative enzymatic activity. Explanatory variables were either root functional traits, soil enzymatic activities or both. Only significant coefficients (p-values <0.05) are reported.

Variable s type	Variables	ß-glucosidase		Cellobiohydrolase	ß-xylanase	*N*-acetyl-ß-glucosaminidase			Phosphomonoesterase		
		Both		Soil	Both	Root	Soil	Both	Root	Soil	Both
Soil enzymes	ßG						-0.164	-0.175		-0.109	-0.106
	CEL						-0.161	-0.171		-0.111	-0.114
	XYL			0.577			-0.138	-0.118		-0.085	
	NAG			0.384			-0.152	-0.156		-0.093	
	PME			0.556			0.149	0.158		0.120	0.173
Root	SRL					0.055		0.060	0.096		0.090
	RMDC										-0.108
	Lignin				-0.046	-0.058		-0.064			-0.063
	HemiCell								0.124		0.104
	Cellulose								0.107		0.091
	Ash								0.161		0.184
	Cortex										-0.067
	Vessel							-0.059	-0.234		-0.238
	C:N								-0.238		-0.233
	N:P	-0.036									
	C:P								-0.126		-0.132
	Al					0.053		0.057			0.069
	Ca								-0.159		-0.151
	Mn							0.065	0.154		0.165
	Na					-0.050		-0.050			0.071

Notes: water saturation, xylem area and potassium content were included but were not significant

Table S2.4 Percentage of mass loss and enzymatic values (µmol MU dry g min^{-1}) found in Sardinilla and Agua Salud. Measurements took at 50, 310, and 485 days of decomposition.

Variable Mean ± SD	Site	Days	*Anacardium excelsum*	*Cedrela odorata*	*Hura crepitans*	*Luehea seemanii*	*Tabebuia rosea*	Five species mixture
Mass loss	Sardinilla	50	24 ±0.4	22 ±1	18 ±2	15 ± 1	17 ± 4	16 ± 2
		310	41 ± 4	38 ± 6	63 ± 2	35 ± 8	43 ± 7	40 ± 4
		485	59 ± 11	60± 21	71 ± 9	43 ± 13	43 ± 1	58 ± 2
	Agua Salud	50	21 ± 2	30 ± 19	38 ± 5	19 ± 1	7 ± 1	25 ± 4
		310	62 ± 6	60 ± 10	79 ± 3	61 ± 8	34 ± 7	58 ± 1
		485	75 ± 4	66 ± 13	90 ± 4	61 ±4	56 ± 5	75 ± 5
ß-Glucosidase (ßG)	Sardinilla	50	39 ± 16	117 ± 53	123 ±46	83 ± 59	25 ± 6	98 ± 13
		310	65 ±38	91 ± 52	152 ± 93	70 ± 30	103 ± 44	102 ± 55
		485	121 ± 32	101 ± 13	175 ± 26	52 ± 29	80 ± 23	109 ± 71
	Agua Salud	50	123 ± 38	136 ± 8	98 ± 32	87 ± 17	185 ± 124	117 ± 29
		310	138 ± 77	97 ± 16	78 ±37	91 ± 76	85 ± 45	103 ± 47
		485	97 ± 45	92 ± 26	53 ± 14	62 ±31	29 ± 13	50 ± 22
Cellobiohydrolase (CEL)	Sardinilla	50	5 ± 3	25 ± 8	32 ± 20	19 ± 16	3 ± 0.3	15 ± 7
		310	24 ± 32	18 ± 13	20 ± 13	18 ± 5	32 ± 17	15 ± 4
		485	32 ± 4	32 ± 15	50 ± 16	18 ± 14	29 ± 7	25 ± 16
	Agua Salud	50	22 ± 12	27 ±6	23 ± 9	19 ± 8	39 ± 33	21 ± 5
		310	31 ± 25	14 ± 5	9 ± 6	17 ±14	13 ± 9	16 ± 8
		485	26 ± 15	24 ± 7	9 ± 5	17 ± 12	10 ± 4	14 ± 10
ß-Xylanase (XYL)	Sardinilla	50	2 ± 1	12 ±5	17 ± 10	6 ± 4	2 ± 1	8 ± 2
		310	3 ± 1	6 ± 2	12 ± 11	8 ± 3	5 ± 1	7 ± 4
		485	12 ± 2	9 ± 3	24 ± 5	8 ± 3	9 ± 3	11 ± 4
	Agua Salud	50	6 ± 3	10 ± 1	9 ± 3	4 ± 1	16 ± 12	8 ± 1
		310	15 ± 5	7 ± 2	10 ± 5	8 ± 7	6 ± 4	11 ± 4
		485	16 ± 7	13 ± 8	7 ± 2	22 ± 26	3 ± 2	7 ± 2
N-acetyl-ß-D-glucosaminidase (NAG)	Sardinilla	50	41 ± 17	87 ± 38	54 ± 33	61 ± 36	21 ± 9	45 ± 28
		310	97 ± 78	49 ± 23	70 ± 33	57 ± 11	52 ± 25	86 ± 42
		485	151 ± 51	71 ± 2	143 ± 32	62 ± 29	65 ± 19	124 ± 73
	Agua	50	140 ± 19	189 ± 25	233 ± 62	98 ± 25	91 ± 45	123 ± 19

	Salud	310	161 ± 70	88 ± 14	127 ± 41	170 ± 82	101 ± 59	85 ± 32
		485	123 ± 63	116 ± 39	87 ± 23	120 ± 42	40 ± 19	84 ± 7
Phosphomonoesterase (PME)	Sardinilla	50	43 ± 6	52 ± 10	173 ± 54	72 ± 38	17 ± 3	93 ± 27
		310	56 ± 22	44 ± 19	205 ± 40	100 ± 106	60 ± 30	68 ± 23
		485	70 ± 25	53 ± 28	187 ± 91	89 ± 87	64 ± 36	118 ± 57
	Agua Salud	50	64 ± 21	92 ± 16	383 ± 89	91 ± 36	84 ± 43	128 ± 14
		310	90 ± 1	116 ± 37	200 ± 56	242 ± 93	66 ± 1	125 ± 68
		485	304 ± 245	216 ± 145	234 ± 90	168 ± 77	77 ± 54	183 ± 141
ßG:NAG	Sardinilla	50	1.5 ± 0.9	1.3 ± 0.5	2.5 ± 0.7	1.4 ± 0.8	1.2 ± 0.2	2.7 ± 1.2
		310	0.8 ± 0.4	2.0 ± 1.4	2.3 ± 1.7	1.2 ±0.5	2.1 ± 0.6	1.2 ± 0.5
		485	0.9 ± 0.5	1.4 ± 0.1	1.3 ± 0.3	0.8 ± 0.3	1.4 ± 0.6	0.9 ± 0.5
	Agua Salud	50	0.9 ± 0.3	0.7 ± 0.1	0.4 ± 0.1	0.9 ± 0.1	1.9 ± 0.6	1.0 ± 0.2
		310	0.8 ± 0.1	1.1 ± 0.2	0.6 ± 0.2	0.5 ± 0.3	0.9 ± 0.1	1.2 ± 0.1
		485	0.8 ± 0.1	0.8 ± 0.3	0.6 ± 0.3	0.5 ± 0.1	0.8 ± 0.1	0.6 ± 0.3
ßG:PME	Sardinilla	50	1.1 ± 0.4	2.6 ± 1.8	0.8 ± 0.4	1.1 ± 0.3	1.5 ± 0.7	1.1 ± 0.2
		310	1.4 ± 1.3	2.4 ± 1.9	0.8 ± 0.5	1.1 ± 0.6	1.8 ± 0.2	1.4 ± 0.5
		485	1.8 ± 0.2	2.3 ± 1.2	1.1 ± 0.7	1.0 ± 0.7	1.6 ± 1.0	1.0 ± 0.8
	Agua Salud	50	2.0 ± 0.3	1.5 ± 0.2	0.3 ± 0.03	1.0 ± 0.4	2.1 ± 0.6	0.9 ± 0.1
		310	1.0 ± 0.1	0.8 ± 0.1	0.4 ± 0.1	0.4 ± 0.2	0.9 ± 0.3	0.7 ± 0.4
		485	0.4 ± 0.2	0.5 ± 0.4	0.2 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.5 ± 0.5
NAG:PME	Sardinilla	50	0.9 ± 0.5	1.8 ± 1.1	0.3 ± 0.2	0.9 ± 0.3	1.3 ± 0.8	0.5 ±0.5
		310	2.2 ± 2.4	1.1 ± 0.1	0.4 ± 0.3	1.1 ± 0.7	0.9 ± 0.3	1.3 ± 0.6
		485	2.5 ± 1.5	1.6 ± 0.8	0.9 ± 0.5	1.3 ± 0.9	1.1 ± 0.2	1.0 ± 0.3
	Agua Salud	50	2.3 ± 0.7	2.1 ± 0.5	0.6 ± 0.2	1.1 ± 0.2	1.1 ± 0.03	1.0 ± 0.2
		310	0.7 ± 0.3	0.7 ± 0.1	1.4 ± 0.3	0.7 ± 0.3	0.8± 0.1	1.0 ± 0.4
		485	0.6 ±0.3	0.6 ± 0.2	0.4 ± 0.3	0.7 ± 0.1	0.6 ± 0.2	0.7 ± 0.5

GENERAL CONCLUSIONS

This thesis focused on coarse root decomposition in tropical forests, given the importance of this process to nutrient cycles globally. To do so, we examined coarse root decomposition from two different ecological perspectives: tree diversity effects at the micro- (within the bag: 0.02 m^2) and meso- (within the plot: 2000 m^2) scales and interactions between root and microbial decomposer community functions during coarse root decomposition. While effects of tree diversity on decomposition rates were not observed at either spatial scale, our results suggest that changes in plant functional composition of tropical forests could alter nutrient cycles via changes in root litter input. In addition, this study provides support for expanding the use of enzymatic activity, typically used in soil science, to decomposition studies in order to illustrate possible underlying mechanisms that determine temporal changes in coarse root decomposition of tropical trees. Enzymatic activity changed with time, elucidating distinct biochemical stages of decomposition, as well as across species and between sites. These results suggest that changes in the functional composition of decomposer communities could influence biochemical processes and that heterogeneous environmental conditions also drive variation in coarse root decomposition. The relationship between fast decomposition rates with high N and P enzymatic production observed in our study could be evidence of the influence of soil nutrients on ecosystem processes, such as P limitation on soils in lowland tropical forests. Hence, in the following sections we make several suggestions to approach, conceptually and experimentally, these ecological concerns.

We did not detect the influence of tree overstory diversity on coarse root decomposition at either very small (0.02 m^2) or medium (2000 m^2) spatial scales. The question whether such an effect could be occurring at an intermediate or even larger spatial scale is still open. Such further experiments could adapt approaches used for evaluating the influence of overstory diversity on tree growth at the tree neighborhood scale, such as Potvin and Dutilleul (2009) and Uriarte et al. (2004), using neighborhood

models. While the model developed by Potvin and Dutilleul (2009) showed a stronger influence of neigbourhood on productivity than tree diversity at meso scales in a tropical tree plantation, Uriarte et al. (2004) found that neigbourhood abundance affected productivity more than neigbourhood diversity in tropical forest. Hence, the use of more flexible spatial statistical approches could improve our ability to detect tree effects on belowground processes and soil communities and to determine the area of influence where diversity effects actually occur.

In this study, we used roots from the 4th and 5th branch orders, which were selected because of their role in the transport and storage of nutrients. Root orders play an important role in C soil retention, as higher root orders are longer lived than lower root orders (Guo et al. 2008) , yet can decompose faster than lower root orders (Fan and Guo 2010, Goebel et al. 2011). Fan and Guo 2010 found that higher orders (5th and 6th) decompose faster than lower orders likely by *i)* their higher nonstructural carbohydrate content and *ii)* greater recalcitrance of lower root orders, due to their close association with mycorrhiza. Still, the mechanisms of root decomposition related to root orders need to be studied. Decomposition rates found in our study (0.4 – 2.6 g year^{-1}) suggests that residence time of belowground carbon could be lower in areas previously disturbed by cattle grazing or agriculture, increasing labile carbon, i.e. mean resistance time of 1 – 2 years approx (Nair et al. 2009). Furthermore, our results emphasize the importance of root traits for predicting decomposition rates. As well as characteristics of a particular decomposition environment appear to modulate the strength of root trait – decomposition relationships.

The strong influence of environmental heterogeneity on root decomposition was reflected in the variation in responses at the micro scale within study plots. As this environmental heterogeneity could be caused by either biotic or abiotic variables (or an interaction of the two), we suggest: 1) including other biotic factors such as understory vegetation composition and diversity as such component could influence decomposition and, subsequently, carbon sequestration, through root exudation, root symbiosis

interactions and specific root litter contributions (Deyn et al. 2008); 2) evaluating the role of diverse groups of decomposers, such as macro-fauna (macro-arthropods and earthworms), which all play a crucial role on leaf decomposition in the tropics (Hättenschwiler et al. 2011); and 3) establishing similar experiments under more controlled conditions to evaluate the influence of specific abiotic factors, such as microenvironment climate conditions, soil nutrient content, soil water saturation, soil percolation, and biotic factors separately. In addition, as our results also showed that soil type modulated variation in root decomposition rates, an experiment using multiple tropical forest sites that encompass a greater range of soil properties and decomposer communities would allow for a more detailed examination of the effects of soil on decomposition in tropical forest, such as priming effects (Fontaine et al. 2003). For these experiments, the use of single-species samples as well as root species mixtures could provide interesting data about the large variation we observed when calculating net diversity effects at the decomposition bag scale.

In our study, the C enzymes used were associated mainly with polysaccharide degradation, at early and intermediate stages of coarse root decomposition. Hence, we suggest including enzymes for lignin degradation, such as phenol oxidase and peroxides, in order to capture the later stages of decomposition. Given the variation observed for the activity of enzymes associated with degradation of C, N and P across study sites, we also suggest examining enzymatic activity during root decomposition across a wider gradient of soil properties. Given that the relationship between root decomposition rates and enzymatic activity between sites may be modulated in part by functionally distinct (i.e. bacterial versus fungal dominated) microbial communities (Wardle et al. 2004), an assessment of microbial diversity in soils and roots during decomposition could help disentangle how soil microbial diversity may influence decomposition and associated nutrient cycles.

Finally, although tree overstory diversity did not influence coarse root decomposition at the spatial scales we tested, we urge caution in interpreting these

results as biodiversity effects may only become evident over longer time scales. A growing network of tree biodiversity experiments globally will provide interesting opportunities in the coming years to study nutrient cycling processes of different aged tree communities across taxonomic and functional diversity gradients.

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